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IN THE UNITED STATES PATENT OFFICE

In re Application of J.K. MEHRA *et al.*
("*Metoprolol Manufacturing Process*")

Serial No. 10/807,221

PETITION UNDER
21 C.F.R. § 1.102(d)

PETITION

This is a Petition under 21 C.F.R. § 1.102(d) to accelerate examination of the captioned application.

ISSUE PRESENTED

Whether examination of this application may be made special?

RELIEF REQUESTED

Applicant respectfully requests examination of this application be made special.

FACTUAL BACKGROUND

The application presents all claims directed to a single invention. If the Office determines that all the claims presented are not obviously directed to a single invention, then the Applicant will make an election without traverse as a prerequisite to the grant of special status.

A pre-examination search has been made. Applicant here submits one copy of each of the references deemed most closely related to the subject matter encompassed by the claims.

Applicant here provides a detailed discussion of the references, which discussion points out with the particularity required by 37 C.F.R. §§ 1.111(b), (c), how the claimed

YOUNG *et al.*

James W. YOUNG *et al.*, WO 92/16197, teaches a method for obtaining an optically pure stereoisomer, (S)-metoprolol. YOUNG summarizes his process at page 9, showing his three-step reaction. In contrast, the first step of the immediate method is to react epichlorhydrin + 4-(2-methoxyethyl)phenol. YOUNG *et al.* begins not with epichlorhydrin, but with a structurally similar compound, glycidol (glycidol is epichlorhydrin with the chlorine replaced with a hydroxyl group). Where the claimed method uses 4-(2-methoxyethyl)phenol, however, YOUNG uses m-Nitrobenzenesulfonyl chloride. *See* YOUNG at page 9, reaction (1).

Britt I. KEDING *et al.*

Britt I. KEDING *et al.*, U.S. 5,034,535, teaches a process for making S-metoprolol via oxazolidin-2-one. KEDING begins with 4-(2-methoxyethyl)phenol, as does the claimed process. KEDING, however, reacts it not with epichlorhydrin, but with (S)-5-hydroxymethyl-3-isopropyloxazolidin-2-one sulfonic acid ester. *See* U.S. '535 at col. 1, line 52.

Sven PALMER *et al.*

Perhaps the nearest prior art to the present invention is the form of Sven PALMER *et al.*, PCT application WO 98/22426 (also published as US Patent No. 6,252,113). PALMER *et al.* discloses reaction of 4-(2-methoxyethyl) phenol and epichlorohydrin in aqueous alkaline conditions. Unlike the claimed process, however, PALMER uses a relatively high temperature - 50° to 70°C temperature - in an attempt to reduce reaction time. PALMER teaches that the high reaction temperature, however, creates an impure product with undesirable contaminants; PALMER thus teaches that the resulting epoxide must be distilled

under high vacuum to improve its purity, and further teaches that the pure epoxide must then be treated with isopropylamine in a solvent such as isopropyl alcohol.

In contrast to the relatively high temperature taught by PALMER, Applicants have disclose and claim the use of a lower temperature range. In contrast to the teachings of PALMER, Applicants have found that the lower reaction temperature works well, and does not need unreasonably-long reaction times.

Gareth Thomas PHILLIPS *et al.*
Gareth Thomas PHILLIPS *et al*, EP 0 193 228, teaches a microorganism-based (fermentation) manufacture of metoprolol, rather than the claimed organic-synthesis process.

Mauro Attilio BERTOLA *et al.*
Mauro Attilio BERTOLA *et al.* EP 0 244 912, teaches a microorganism-based (fermentation) manufacture of metoprolol, rather than the claimed organic-synthesis process.

Gert A. RAGNARSSON *et al*
Gert A. RAGNARSSON *et al.*, U.S. 4,942,040, teaches a controlled-release formulation of metoprolol and felodipine. In contrast to the immediate claims, RAGNARSSON *et al.* fails to teach a method to make metoprolol.

Joseph M. RIBALTO BARA *et al.*
Joseph M. RIBALTO BARA *et al.*, US Letters Patent No. 5,082,969 (Spanish patent No. ES 2,011,584) describes a process for manufacturing metoprolol, where 4-(2-methoxyethyl) phenol and epichlorohydrin are reacted in aqueous alkaline conditions. Unlike the claimed process, however, RIBALTO BARA teaches the use of a relatively low reaction temperature – a range of 0° to 25°C – rather than the higher reaction temperature taught by the Applicants. In contrast to RIBALTO BARA, who teach that the reaction takes 15 to 20 hours to complete, the Applicants teach a shorter potential reaction time, while avoiding the

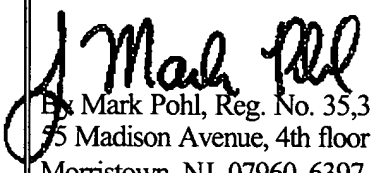
formation of excessive amounts of undesirable reaction by-products. RIBALTO BARA teaches that even at the lower temperature, some residual reaction by-products exist, and that to remove these, the organic phase consisting of epoxide is separated, washed with water and used as such for reaction with large excess of isopropylamine in an aqueous media at 0° to 30°C temperature.

SUMMARY

The forgoing does not provide an exhaustive enumeration of every difference between each reference and the pending claims, but does provide information adequate to show that no reference bars the pending claims.

Enclosed find (i) a photocopy of each reference discussed herein,[1] and (ii) a FEE TRANSMITTAL FORM and the appropriate filing fee for this paper.

Respectfully submitted,
Pharmaceutical Patent Attorneys LLC



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[1] In addition to the above, Applicant believes that Polish Patent PL 158,497 describes a process wherein 4-(2-methoxyethyl) phenol and epichlorohydrin are reacted at 20° to 80°C temperature for 3 hours under aqueous alkaline conditions. The epoxide so formed is reacted with large excess of isopropyl amine (medium as well as reactant) to yield metoprolol base. The undersigned has not, however, been able to locate any record of PL158497 on the European Patent Office database, and accordingly does not provide a copy here.

subject matter is patentable over the references. In organizing this detailed discussion, Applicant first briefly reviews the pending claims, and then discusses the various references.

The Pending Claims

This invention relates to an improved process to manufacture metoprolol.

The immediate application claims a method of manufacture, and the counterpart product-by-process:

> 1. A process for obtaining an aryloxypropanolamine of the chemical name 1-[4-)2-methoxyethyl)-phenoxy]-3-[(1-methylethyl)amino]-2-propanol ... comprising:
>
> A) combining 4-(2-methoxyethyl)phenol with epichlorhydrin;
>
> B) reacting said combination of 4-(2-methoxyethyl)phenol and epichlorhydrin in an alkaline aqueous medium;
>
> C) extracting and washing the organic phase reaction product of Step B with water at pH 7.5 $\pm$0.5; and
>
> D) obtaining a crude reaction product comprising 3-[4-(2-methoxyethyl)phenoxy]-1,2-epoxypropane;
>
> E) combining said 3-[4-(2-methoxyethyl)phenoxy]-1,2-epoxypropane with isopropanolamine;
>
> F) reacting said combination of 3-[4-(2-methoxyethyl)phenoxy]-1,2-epoxypropane and isopropanolamine in an aqueous medium at a temperature about 30 °C, to obtain 1-[4-)2-methoxyethyl)-phenoxy]-3-[(1-methylethyl)amino]-2-propanol.

The prior art identified by the Applicant includes the following references:

Applicant Has Identified No Reference
Which Bars the Claims Under U.S. Law

Applicant has searched published international applications which mention the word "metoprolol" in the title. The most relevant references are the following.

Curt H. APPELGREN *et al*

Curt H. APPELGREN *et al.*, U.S. 5,081,154, teaches "a previously unknown compound, viz. metoprolol succinate." Id. at col. 2, lines 4. In contrast to the immediate claims, however, APPELGREN *et al.* fails to teach how to make metoprolol.

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(54) **A process for the preparation of R and S-2,2-R1,R2-1,3-dioxolane-4-methanol.**

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TETRAHEDRON REPORT NUMBER 195, vol. 42, no. 2, 1986, pages 447-488, Pergamon Press Ltd, Oxford, GB; J. JURCZAK et al.: "(R)- and (S)-2,3-0-isopropylideneglyceraldehyde in stereoselective organic synthesis"

The file contains technical information submitted after the application was filed and not included in this specification

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Description

The present invention relates to a process for the preparation of 2,2-R_1,R_2-1,3-dioxolane-4-methanol consisting predominantly of or substantially completely of *R*-isomer.

R-2,2-dimethyl-1,3-dioxolane-4-methanol is an important starting material for the preparation of agricultural and pharmaceutical products, see for example J. Jurczak et al., Tetrahedron vol. 42, no. 2, pp. 447—488 (1986).

In recent years, *R*-2,2-dimethyl-1,3-dioxolane-4-methanol has become of interest as an important starting compound for the preparation of many biologically active products and especially for the preparation of chiral drugs. The preparation of biologically active products in optically pure form using chiral starting materials is very advantageous, enabling precise planning and efficient realization of synthetic pathways. *R*-2,2-dimethyl-1,3-dioxolane-4-methanol is an important example of C_3-synthon and is used as starting compound for the preparation of many other C_3-synthons which are widely applied in organic synthesis as a chiral building block. For example, *R*-2,2-dimethyl-1,3-dioxolane-4-methanol can be used in the synthesis of other chiral synthons, monosaccharides, their derivatives and other polyhydroxyl systems, and biologically active products of more complex structure. Examples of the syntheses of more complex structures are the preparation of β-blockers or optically pure β-lactam systems.

Therefore there is still a great need for a process, usuable on an industrial scale giving rise to economically attractive yields for the stereoselective preparation of the *R*-stereoisomer of 2,2-dimethyl-1,3-dioxolane-4-methanol. The present invention provides such a process. It has been found that the stereoselective preparation of the *R*-stereoisomer can be advantageously carried out using micro-organisms or substances derived therefrom. Moreover it has been found that the *S*-stereoisomer of 2,2-R_1,R_2-1,3-dioxolane-4-methanol may be advantageously converted into *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboyxlic acid using these micro-organisms or substances derived therefrom.

The present invention provides a process for the preparation of 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer wherein R_1 and R_2 are H or alkyl groups, optionally substituted or branched, or wherein R_1 and R_2 together with the carbon atom to which they are attached form a carbocyclic ring, optionally substituted, which comprises subjecting a mixture of *R*- and *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol to the action of a micro-organism or substances derived therefrom having the ability for stereoselective consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol for a period of time such that *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol in the mixture is consumed to give a 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer. Suitably at least 80 wt%, preferably 90 wt% and more preferably substantially all of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is consumed, to give 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer or consisting of substantially pure R-isomer. The 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer is at least partly isolated and/or used as starting material for the preparation of other optically active compounds. Advantageously R_1 and R_2 are alkyl groups containing less than 6 carbon atoms or the carbocyclic ring contains less than 8 carbon atoms. Preferably R_1 and R_2 are identical. In this way no extra asymmetry is brought in the compounds. More preferably R_1 and R_2 are an alkyl group containing 1—3 carbon atoms or together with the carbon atom to which they are attached form a carbocyclic ring containing 5 or 6 carbon atoms.

As hereinbefore described the mixture of *R*- and *S*-isomers of 2,2-R_1,R_2-1,3-dioxolane-4-methanol may be converted into 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in *R*-isomer. In this way only part of the mixture can be used in further reactions, for example starting with a 50 wt% R- and 50 wt% S-mixture only half of the 2,2-R_1,R_2-1,3-dioxolane-4-methanol becomes available in a useful way. According to an embodiment of the invention, the *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is advantageously converted into *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboxylic acid.

In this way not only the preparation of 2,2-R_1,R_2-1,3-dioxolane-4-methanol (enriched in *R*-isomer) is possible, but at the same time 2,2-R_1,R_2-1,3-dioxolane-4-carboxylic acid enriched in *R*-isomer may be obtained. In this way substantially all the *R*- and *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol may be separated and may be used. The 2,2-R_1,R_2-1,3-dioxolane-4-carboxylic acid enriched in *R*-isomer may be used as starting compound for the preparation of many biologically active products or may be converted to the 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in *S*-isomer, which is an important starting compound as well.

By the term "micro-organisms having the ability for stereoselective consumption" is meant, for example, bacteria, yeasts, fungi. Suitable bacteria are, for example, micro-organisms belonging to the genus *Nocardia*, to the genus *Rhodococcus*, to the genus *Mycobacterium* or to the genus *Corynebacterium*.

Also mciro-organisms, which have obtained the ability for consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol through the introduction of novel genetic material are embodied by the term "micro-organism, having the ability for stereoselective consumption".

This can be accomplished by transferring the cloned gene encoding a polypeptide responsible for the stereoselective consumption, an enzyme, from any of the screened micro-organisms to another micro-organism, particularly to *Escherichia coli*. Other micro-organisms may be belonging to the genus *Saccharomyces, Kluyveromyces, Bacillus, Nocardia, Rhodococcus, Escherichia* and *Corynebacterium*. Cloned genes may be selected for their ability to encode an enzyme capable of consuming of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol, preferably *S*-2,2-dimethyl-1,3-dioxolane-4-methanol. Alternatively they may be

selected by cross-hybridization with an already selected gene encoding an enzyme for the stereoselective consumption.

The micro-organisms may advantageously be immobilized for example on a polymer gel. This can be done with living cells, killed cells and/or resting cells, but alternatively with suitable enzymes derived therefrom, which may be purified to a certain extent if a higher specific activity is needed.

Therefore by the term "micro-organisms or substances derived therefrom" is meant the micro-organisms, killed, alive or resting, and extracts therefrom, optionally concentrated or purified. For example, enzymes optionally in combination with, for example, artificial or natural co-factors, may be used. No fermentatively active cells may be used for the consumption of the *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol. It is found that enzymes derived from the cells or killed cells may consume the S-isomer under suitable conditions. The micro-organisms or substances derived therefrom may be used several times and are active for at least 2 weeks. Even without co-substrate (for example glucose) the micro-organisms may remain active. The enrichment in *R*-isomer of *R*-2,2-R_1,R_2-1,3-dioxolane-4-methanol may take take place in suitable buffers (for example 3-(N-morpholino)propane sulfonic acid, tris(hydroxymethyl)aminomethane or potassium phosphate) as well as in physiological salt solutions. After being stored the induced cells are found to be directly capable to perform the enrichment in *R*-isomer.

More particularly the micro-organism for the consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol include cultures of *Rhodococcus equi, Rhodococcus rhodochrous, Rhodococcus erythropolis, Corynebacterium equi, Corynebacterium alkanum* (a sample of this species is deposited with the ATCC under the accession number of 21194), *Corynebacterium hydrocarboclastus* (a sample of this species is deposited with the ATCC under the accession number of 15108), *Corynebacterium* species T 1300 (a sample of this species is deposited with the CBS under the accession number of 267.87), *Corynebacterium species DS 5122* (a sample of this species is desposited with the CBS under the accession number of 265.87), *Nocardia erythropolis, Nocardia corallina* (a sample of this species is deposited with the ATCC under the accession number of 31338), *Nocardia canicruria* (a sample of this species is deposited with the ATCC under the accession number of 31548), *Nocardia paraffinae* (a sample of this species is deposited with the NCIB under the accession number of 11277), *Nocardia species DS 5123* (a sample of this species is deposited with the CBS under the accession number of 266.87), *Nocardia aurantia* (a sample of this species is deposited with the NCIB under the accession number of 9557), *Nocardia calcarea* (a sample of this species is deposited with the NCIB under the accession number of 8863), *Nocardia cathaarde* T 985 (a sample of this species is deposited with the CBS under the accession number of 268.87), *Nocardia globerula* (a sample of this species is deposited with the NCIB under the accession number of 9159), *Nocardia ragosa* (a sample of this species is deposited with the NCIB under the accession number of 8926) and *Mycobacterium rhodochrous* (a sample of this species is deposited with the NCIB under the accession number of 11061).

Advantageously cultures of the *Rhodococcus equi* include cultures of species *Rhodococcus equi* (a sample of this species is deposited with the IFO under the accession number of 03730) and species *Rhodococcus equi* (a sample of this species is deposited with the NCIB under the accession number of 12035). Advantageously cultures of the *Rhodococcus rhodochrous* include cultures of species *Rhodococcus rhodochrous* (a sample of this species is deposited with the NCIB under the accession number of 9703) and species *Rhodococcus rhodochrous* (a sample of this species is deposited with the ATCC under the accession number of 21197). Advantageously cultures of the *Rhodococcus erythropolis* include cultures of species *Rhodococcus erythropolis* SCL 38—2 (a sample of this species is deposited with the CBS under the accession number of 179.86 on April 18, 1986), species *Rhodococcus erythropolis* SCL 38—2R (a sample of this species is deposited with the CBS under the accession number of 180.86 on April 18, 1986) and species *Rhodococcus erythropolis* SCL 38—2S (a sample of this species is deposited with the CBS under the accession number of 181.86 on April 18, 1986). Advantageously cultures of the *Corynebacterium equi* include cultures of species *Corynebacterium equi* A 2362 (a sample of this species is deposited with the CBS under the accession number of 264.87) and species *Corynebacterium equi* A 2431 (a sample of this species is deposited with the CBS under the accession number of 263.87). Advantageously cultures of the *Nocardia erythropolis* include cultures of species *Nocardia erythropolis* T 487 (a sample of this species is deposited with the NCIB under the accession number of 9158), species *Nocardia erythropolis* (a sample of this species is deposited with the DSM under the accession number of 743) and species *Nocardia erythropolis* (a sample of this species is deposited with the ATCC under the accession number of 4277).

The *R*-2,2-R_1,R_2-1,3-dioxolane-4-methanol produced according to the present invention may be used as a starting material for the production of other optically active compounds. An important possibility is the production of chiral drugs.

It will be appreciated by everyone skilled in the art that any suitable process may be used to convert *S*- and *R*-2,2-R_1,R_2-1,3-dioxolane-4-methanol in the optically active compounds.

Examples which illustrates the use of *S*- and *R*-2,2-dimethyl-1,3-dioxolane-4-methanol for the preparation of optically active compounds are known from, for example, J. Jurczak et al, Tetrahedron report number 195, Tetrahedron *42* (1986), p. 447—488 and the Technical Information Bulletin 225, September 1983 of Janssen Chimica (Belgium).

The optically active compounds may be used in pharmaceutical or agricultural products.

According to a preferred embodiment of the process of the present invention a micro-organism having the ability for stereoselective consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol has to be cultured for about 0.5 to 10 days, whereafter the cells of the micro-organisms are suspended in a liquid nutrient medium and the mixture of *R*- and *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is subjected to the action of the cells.

After the abovementioned cultivation for about 0.5 to 10 days the cells may be isolated from the culturing medium before suspending the cells in the liquid nutrient medium.

To grow the micro-organism used for the selective consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol, ordinary culture medium containing an assimilable carbon source (for example glucose, lactate, sucrose, etc.), an assimilable nitrogen source (for example ammonium sulphate, ammonium nitrate, ammonium chloride, etc.), with an agent for an organic nutrient source (for example yeast extract, malt extract, peptone, meat extract, etc.) and an inorganic nutrient source (for example phosphate, magnesium, potassium, zinc, iron and other metals in trace amounts) may be used.

A Jap medium optionally enriched with one or more ingredients may be used as a suitable culture medium. A Jap medium of the following composition may be used: soybean flour (30 g/l), sodium nitrate (7.5 g/l), ferrous sulphate.$7H_2O$ (0.28 g/l), sodium citrate (6 g/l) and fructose (12.5 g/l), the pH adjusted to 7.2. Before use the medium is conveniently sterilized for 20 minutes at 120°C.

Another preferred culture medium is a YPD-medium optionally enriched with one or more ingredients. A YPD medium consisting of 20 g/l bactopeptone, 10 g/l yeast extract and 20 g/l glucose may be used. Before use, the medium is conveniently sterilized for 30 minutes at 110°C.

Another preferred culture medium is a GYMB-medium optionally enriched with one or more ingredients. A GYMB-medium of the following composition may be used: glucose (10 g/l), peptone (5 g/l), yeast extract (3 g/l), malt extract (3 g/l), which is conveniently sterilized for 20 minutes at 120°C before use.

Another preferred culture medium is a medium 3310 optionally enriched with one or more ingredients. A medium 3310 of the following composition may be used: $(NH_4)_2SO_4$ (10 g/l), NaCl (1 g/l), $MgSO_4.7H_2O$ (1 g/l), K_2HPO_4 (5 g/l), $MnSO_4.3H_2O$ (2.5 mg/l), $ZnSO_4.7H_2O$ (2.5 mg/l), $FeSO_4.7H_2O$ (2.5 mg/l), $CaCl_2$ (12.5 mg/l), $CuSO_4.5H_2O$ (0.75 mg/l), $CoCl_2.6H_2O$ (0.75 mg/l), H_3BO_3 (0.25 mg/l), $Na_2MoO_4.2H_2O$ (0.275 mg/l), KI (0.5 mg/l), Capantothenate (0.8 mg/l), inositol (4 mg/l), nicotinic acid (0.8 mg/l), thiamin.HCl (0.8 mg/l), pyridoxin.HCl (0.8 mg/l), p-aminobenzoic acid (0.4 mg/l), riboflavin (0.4 mg/l), folic acid (0.02 mg/l), biotin (4 µg/l). Before use the medium is conveniently sterilized for 20 minutes at 120°C. Thereafter glucose (for example 50 g/l) sterilized for 30 minutes at 110°C is added.

Another preferred culture medium is a skimmed milk medium optionally enriched with one or more ingredients, for example, a skimmed milk medium containing: 10% skimmed milk from skimmed milk powder, which is conveniently sterilized for 30 minutes at 110°C before use.

Examples of enrichments to the skimmed milk medium include 0.5% lactate or PSIII salts or combinations thereof. PSIII salt medium of the following composition can be used: potassium dihydrogen phosphate (2.1 g/l), ammonium monohydrogen phosphate (1.0 g/l), ammonium sulphate (0.9 g/l), potassium chloride (0.2 g/l), sodium citrate (0.29 g/l), calcium sulphate.$2H_2O$ (0.005 g/l), magnesium sulphate.$7H_2O$ (0.2 g/l), ammonium ferrous sulphate.$6H_2O$ (2.5 mg/l), zinc sulphate.$7H_2O$ (0.5 mg/l), manganese chloride.$4H_2O$ (0.3 mg/l), copper sulphate.$5H_2O$ (0.15 mg/l), cobalt chloride.$6H_2O$ (0.15 mg/l), ortho-boric acid (0.05 mg/l), sodium molybdate.$2H_2O$ (0.055 mg/l) and potassium iodide (0.1 mg/l), the pH was adjusted at 6.8. Before use the PSIII salt medium is conveniently sterilized for 30 minutes at 120°C.

A temperature of 0 to 45°C and a pH of 3.5 to 9 is preferably maintained during the growth of the micro-organism. Preferably the micro-organism is grown at a temperature of 20 to 37°C and at a pH of 5 to 9. The aerobic conditions required during the growth of the micro-organisms can be provided by any of the well-established procedures, provided that the supply of oxygen is sufficient to meet the metabolic requirement of the micro-organisms. This is most conveniently achieved by supplying oxygen, suitably in the form of air and optionally at the same time shaking or stirring the reaction liquid. During the consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol by the micro-organism, the micro-organism might be in a growing stage using an abovementioned ordinary culture medium.

During the consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol by the micro-organism, an ordinary culture medium may be used containing an assimilable carbon source when required (for example glucose, lactate, sucrose, etc.), an assimilable nitrogen source when required (for example ammonium sulphate, ammonium nitrate, ammonium chloride, etc.), with an organic nutrient source when required (for example yeast extract, malt extract, peptone, meat extract, etc.) and an inorganic nutrient source when required (for example phosphate, magnesium, potassium, zinc, iron and other metals in trace amounts).

Advantageously, during the enrichment of *R*-isomer, a Jap medium, a YPD medium, a Medium 3310 or a skimmed milk medium (as described above) optionally enriched with one or more ingredients can be used. Preferably a GYMB medium (as described above) optionally enriched with one or more ingredients is used.

The micro-organism can be kept in the non-growing stage, for example, by exclusion of the assimilable carbon source or by exclusion of the nitrogen source. A temperature of 0 to 45°C and a pH of 3.5 to 9 can be maintained during this stage.

Preferably the micro-organisms are kept at a temperature of 20 to 37°C and a pH of 5 to 9. The aerobic conditions required during this stage can be provided according to any of the well-established procedures,

provided that the supply of oxygen is sufficient to meet the metabolic requirement of the micro-organisms. This is most conveniently achieved by supplying oxygen, suitably in the form of air and optionally, at the same time, shaking or stirring the reaction liquid. The *R*- and any remaining *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol after transformation as mentioned above, can then be recovered and purified according to any of the procedures known *per se* for such products.

The micro-organisms can be kept on agar slants, frozen in 50% glycerol or lyophilized. If required, precultures of these micro-organisms can be made according to any of the well-established procedures, for example, the micro-organisms can be incubated in bouillon or in BHI (Brain Heart infusion) for 24 hours at 30°C in a rotary shaker. A bouillon medium of the following composition can be used: Lab Lemco L 29 (meat extract, Oxoid®) (9 g/l), Bactopeptone (10 g/l) and sodium chloride (5 g/l), the pH adjusted to 7.6. Before use this medium is conveniently sterilized for 20 minutes at 120°C.

A BHI (brain-heart infusion) medium containing 0.037 g/l BHI (Oxoid®), the pH adjusted to 7.0, can be used. Before use this medium is conveniently sterilized for 20 minutes at 120°C.

The present invention will be further illustrated with reference to the following Examples.

Example 1

Rhodococcus equi NCIB 12035 was grown for 48 hours at 30°C, in several 500 ml baffle flasks containing 100 ml of 10% skimmed milk medium. Thereafter 120 µl of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was added to each flask and incubated further for 24, 48 and 96 hours. At each time indicated 3 flasks (300 ml culture) were extracted using a continuous extraction procedure. The extracts were purified on a silicagel column and the optical rotation was measured in a polarimeter in 1 or 10 cm pathlength cell (volume 0.5—5 ml) maintained at 22°C and ethanol as solvent.

Rotations were recorded at 589 nm (Sodium D-line).

The enantiomeric distributions were measured with NMR using an Europium(HFC)$_3$ shift reagent.

The results are presented in Table 1 here below.

Table 1

Incubation time (h)	Quantity 2,2-dimethyl-1,3-dioxolane-4-methanol (mg)	(α_D)	Enantiomer distribution %R	%S
24	95.5	- 5	75	25
48	61.1	- 9.7	100	0
96	54.0	-10.1	100	0

Example 2

Rhodococcus erythropolis SCL 38—2 (CBS 179.86) was grown as described in Example 1 and after the addition of 120 µl of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol further incubated for 12, 24 and 48 hours. Extraction, purification and measurements of the 2,2-dimethyl-1,3-dioxolane-4-methanol were made as described in Example 1.

The results are presented in Table 2 here below.

Table 2

Incubation time (h)	Quantity 2,2-dimethyl-1,3-dioxolane-4-methanol (mg)	(α_D)	Enantiomer distribution %R	%S
12	93.6	- 8.8	93	7
24	77.8	-10.4	100	0
48	69.1	- 9.8	100	0

Example 3

Rhodococcus erythropolis SCL 38—2 (CBS 179.86) was first grown for 48 hours at 30°C in several 100 ml baffle flasks containing 25 ml 10% skimmed milk medium after which *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was added to the cultures. To each series of the flasks was added a different starting concentration of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol of 1.2, 2.4, 4.8 and 9.6 g/l respectively. At certain time-points samples of a flask of each series was taken, extracted and analysed. It appeared that up to 4.8 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol, half of the added amount is consumed within 48 hours. At 9.6 g/l it take more than 96 hours before half of the *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol is consumed. Since it was assumed that most of the 2,2-dimethyl-1,3-dioxolane-4-methanol consumed would have the *S*-configuration, a new experiment based on the experience obtained aboved was set up in order to prepare larger quantities of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol.

A total of 5.76 g *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was divided over twelve 500 ml baffle flasks containing 100 ml 10% skimmed milk in which the *Rhodococcus erythropolis* SCL 38—2 had grown for 48 hours. The cultures were then further incubated at 30°C for 96 hours. From the total volume of ca. 1.2 l the remaining 2,2-dimethyl-1,3-dioxolane-4-methanol was extracted and purified as described in Examples 1 and 2.

An amount of 1.5 g 2,2-dimethyl-1,3-dioxolane-4-methanol was isolated and NMR measurements showed it to be of the *R*-configuration, 100% enantiomer pure.

Example 4

In an experiment *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) was grown at 30°C for 48 hours in three 2 l baffle flasks containing 500 ml 10% skimmed milk. A total of 7.2 g *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was divided over the cultures and incubated further for 96 hours. Extraction and purification of the remaining 2,2-dimethyl-1,3-dioxolane-4-methanol was performed as described in Examples 1, 2 and 3.

An amount of 1.4 g *R*-2,2-dimethyl-1,3-dioxolane-4-methanol was recovered, according to NMR measurements 100% enantiomer pure.

Example 5

The three variant forms of *Rhodococcus erythropolis* SCL 38—2, SCL 38—2S and SCL 38—2R, respectively, were each grown in twice 100 ml skimmed milk as described in example 1 for 48 hours. The biomass dry weights were determined and found to be equal for all three forms. The cultures were divided in portions of 30 ml and put in 100 ml baffle flasks. In this way the cultures of each form were split in five different cultures of 30 ml. 60 µl of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol were added to these cultures and incubated further. At different time points, (see Table 3), one culture of each form was taken, extracted and the remaining 2,2-dimethyl-1,3-dioxolane-4-methanol was analysed. The enantiomeric distribution was determined by formation of diastereoisomers with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) which were separated on a chiral complexation column. No clear differences were found between the three forms.

Table 3

	SCL 38—2R				SCL 38—2S				SCL 38—2			
assay time	quantity left over g/l		enantiomeric distribution		quantity left over g/l		enantiomeric distribution		quantity left over g/l		enantiomeric distribution	
hour	R	S	%R	%S	R	S	%R	%S	R	S	%R	%S
0	0.55	0.55	50	50	0.57	0.54	50	50	0.55	0.55	50	50
3	0.57	0.57	50	50	0.55	0.55	50	50	0.54	0.53	50	50
6	0.57	0.49	54	46	0.57	0.52	53	47	0.56	0.47	55	45
8	0.57	0.38	60	40	0.57	0.39	59	41	0.57	0.35	62	38
10	0.46	0.00	100	0	0.49	0.00	100	0	0.45	0.00	100	0

Example 6

This examples describes the preparation of S-metoprolol, a beta-blocker from *R*-2,2-dimethyl-1,3-dioxolane-4-methanol. This example demonstrates the possibility of using *R*-2,2-dimethyl-1,3-dioxolane-4-methanol as a starting material for the synthesis of other optically active materials.

Preparation of *S*-2,2-dimethyl-4-methanesulphonyloxymethyl)-1,3-dioxolane from *R*-2,2-dimethyl-1,3-dioxolane-4-methanol

A mixture of 1.2 ml (15.5 mmole) of methanesulphonyl chloride and 4 ml of methylene chloride was

added in 5 min. to a stirred and cooled mixture of 7 ml of methylene chloride, 2.6 ml (18.7 mmole) of triethylamine and 1.81 g (13.7 mmole) *R*-2,2-dimethyl-1,3-dioxolane-4-methanol (which was optically pure by NMR). This R-isomer was prepared by the procedure described in Examples 1—5.

Stirring was continued at 22°C for 1½ hours and next more methanesulphonyl chloride (0.15 ml) and triethylamine (0.5 ml) were added. After standing in the refrigerator overnight the mixture was shaken with methylene chloride and 1 N sodium bicarbonate solution. The organic layer was washed with 1 N sodium bicarbonate solution, filtered and evaporated to give 2.78 g of *S*-2,2-dimethyl-4-(methanesulphonyloxymethyl)-1,3-dioxolane as brown oil, which was used as such.

Preparation of *S*-2,2-dimethyl-4-[p-(2-methoxyethyl)-phenyloxymethyl]-1,3-dioxolane from *S*-2,2-dimethyl-4-(methanesulphonyloxymethyl)-1,3-dioxolane

p-(2-methoxyethyl)-phenol (2.2 g, 14.47 mmole) was added to a cooled (to keep the reaction mixture below 100°C) and stirred mixture of 10 ml of dry N,N-dimethylformamide and 0.38 g (15.8 mmole) of sodium hydride.

Next 2.78 g of crude *S*-2,2-dimethyl-4-(methanesulphonyloxymethyl)-1,3-dioxolane were added and the mixture was stirred and heated in an oil bath of 100°C for 1½ hours.

After cooling to 22°C the mixture was poured into 1 M sodium bicarbonate and extracted with ether. The ether extract was washed with 1 N sodium hydroxide, 1 M sodium bicarbonate, brine, dried with magnesium sulphate, filtered and evaporated to give 3.20 g of *S*-2,2-dimethyl-4-[p-(2-methoxyethyl)-phenyloxymethyl]-1,3-dioxolane as an oil, which was used as such.

Preparation of *S*-3-[p-(2-methoxyethyl)-phenoxy-1,2-propanediol from *S*-2,2-dimethyl-4-[p-(2-methoxyethyl)-phenyloxymethyl]-1,3-dioxolane

A mixture of 3.20 g of crude *S*-2,2-dimethyl-4-[p-(2-methoxyethyl)-phenyloxymethyl]-1,3-dioxolane, 15 ml of methanol, 10 ml of water and 0.5 ml of 36% hydrochloric acid was stirred at 22°C for 1 hour. After the addition of another 0.5 ml of 36% hydrochloric acid stirring was continued for ½ hour and next 10 ml of 1 M sodium carbonate were added and the mixture concentrated in vacuum.

The residue was extracted with ether and the extract was washed with brine, filtered and evaporated to give 2.60 g of the crude title compound as an oil, which was used as such.

Preparation of *R*-1-bromo-2-acetoxy-3-[p-(2-methoxyethyl)-phenyloxy]-propane from *S*-3-[p-(methoxyethyl)-phenoxy]-1,2-propanediol

A mixture of 2.6 g of crude *S*-3-[p-(methoxyethyl)-phenyloxy]-1,2-propanediol and 10 ml of 33% hydrobromic acid in acetic acid was stirred at 22°C for 1½ hours.

Next the mixture was poured into 100 ml of 1 M sodium carbonate and extracted with diethyl ether. The extract was washed with 1 M sodium carbonate, brine, dried with magnesium sulphate, filtered and evaporated to give 3.65 g of crude title compound as an oil, which was used as such.

Preparation of *S*-p-(2-methoxyethyl)phenyl glycidyl ether from R-1-bromo-2-acetoxy-3-[p-(2-methoxyethyl)-phenyloxy]-propane

A mixture of 3.65 g of crude *R*-1-bromo-2-acetoxy-3-[p-2-methoxyethyl)-phenyloxy]-propane, 65 ml of dry 1,2-dimethoxyethane and 2.2 g (39.3 mmole) of powdered potassium hydroxide was stirred at 22°C for 20 hours. After standing in the refrigerator for two days 1 M sodium bicarbonate (50 ml) was added and the mixture was concentrated in vacuum and extracted with ether.

The extract was washed with 1 M sodium bicarbonate and brine and dried with magnesium sulphate.

After filtration the filtrate was evaporated to give 2.04 g of an oil. This was purified over a Merck Fertigsäule C packed silica gel column with ether/hexane = 1:1 and ether/ethanol = 20:1 as the eluent. The fractions containing the epoxide were combined and evaporated to give 1.31 g of the title compound as an oil.

Preparation of *S*-1-isopropylamino-3-[p-(2-methoxyethyl)-phenyloxy)-2-propanol (*S*-metoprolol) from *S*-p-(2-methoxyethyl)phenyl glycidyl ether

A mixture of 1.31 g (6.3 mmole) of *S*-p-(2-methoxyethyl)phenyl glycidyl ether, 10 ml of ethanol and 5 ml of isopropylamine was heated in a water bath of 55°C for 1½ hours.

The mixture was evaporated to give 1.67 g of title compound as a solid. After derivatisation of a sample with the acid chloride of (+)-α-methoxy-α-trifluoromethyl-phenyl acetic acid the resulting amide showed in NMR the presence of only 0.7 wt% of the R-isomer.

Example 7

Rhodococcus erythropolis SCL 38—2 (CBS 179.86) was grown in a 3 l fermentor in 1.5 l of 5x concentrated GYMB medium at an air supply of 90 l/h. The medium was stirred and kept at a pH of 6.8 to 7.2. As inoculum 15 ml of a 48 h old *Rhodococcus erythropolis* culture grown on a normal GYMB medium was used. After 65 h of growth the culture, having the optical density at 600 nm of 37 in a 1 cm pathlength cell of LKB Ultrospec 4050 was incubated with 15 g *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 4 h. Subsequently the pH was increased to 7.5 and kept constant by a 4N NH_4OH supply during a feed

containing 8 M *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol (feed rate 2.2 g/h). The concentrations of *R*- and *S*-2,2-dimethyl-1,3-dioxolane-4-methanol were assayed by elution of 3 ml fermentation broth over an Exterlut-3 column (Merck nr. 15372). At least 5 min. later the column was eluted with 12 ml ethyl acetate. This procedure gave a quantitative extraction of 2,2-dimethyl-1,3-dioxolane-4-methanol. Diastereoisomers of this alcohol were formed with BSTFA (see Example 5). The ratio of both enantiomers could be determined by use of a chiral complexation column (Table 4).

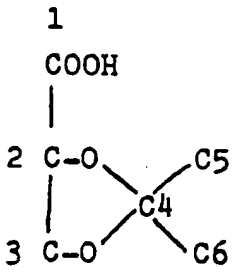
TABLE 4

The enantiomeric excess of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol in a fermentation broth of *Rhodococcus erythropolis* supplied with a feed of a racemic mixture of this compound

h after start of the feed	ee of the R enantiomer
0	0.43
20	0.94
26	0.97
29	0.97

ee = enantiomeric excess

The feed was continued during 29 h. At that moment the broth contained 24.3 g/l of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol and 0.37 g/l of the S-enantiomer. During the feed a constant production of acid was observed. The produced acid was identified as 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid by means of NMR. The 13C NMR spectrum exhibits the following signals:



	δ (ppm/TMS)
C1	180.11
C2	77.64
C3	69.49
C4	113.00
C5, C6	27.43, 27.03

TMS = tetra methylsilane

Example 8

A 6 l culture of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) was grown on medium 3310 in a 10 l fermentor. Cells were cultured at 30°C, the medium was stirred, air was supplied (360 l/h) and the pH was regulated between 6.8 and 7.2 by the addition of 4N H_2SO_4 or 4N NH_4OH. The culture reached an optical density at 600 nm of 60 and at that moment 10 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol were added. After complete consumption of the *S*-enantiomer again 10 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol were added. The final alcohol obtained had an enantiomeric excess of the *R*-enantiomer of 0.97. The final broth (5.5 l) contained 47.0 g of 2,2-dimethyl-1,3-dioxolane-4-methanol.

Example 9

Shake flasks of 2 l, containing 500 ml of GYMB medium were inoculated with 10 ml of a 24 h culture of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) grown on BHI medium in shake flasks. Cells were grown during 48 h at 30°C. The culture was induced by incubation in 5 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 5.5 h. Thereafter the cells were harvested by centrifugation and the pellets were resuspended in various buffers resulting in a concentration with a factor of 6 (see Table 5).

A volume of 100 ml of each suspension was transferred to a 500 ml baffled shake flask and *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was added at various time points. The concentrations of *R*- and *S*-2,2-dimethyl-1,3-dioxolane-4-methanol were assayed in an ethyl acetate extract of the suspension. The extraction yield of this extraction was found to be 40%. The results presented in Table 5 show that resting cell suspensions can produce large quantities of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol.

TABLE 5

Scheme of additions of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol and measured excess of the R enantiomer during incubation of cell suspensions in the presence of various buffers

Suspensions	time (h)										final conc. g/l[6]
	0	24	42	50	73	90	162	167[4]	194	236	
a. 1M MOPS[1] pH = 7.5 additions	3g	-	-	2.1g	-	-	-	0.25g	0.9g	-	
e.e.	n.m.	0.32	0.95	n.m.	0.60	0.68	0.90	0.90	n.m.	0.79	30.3
b. 1M potassium phosphate pH = 7.5 additions	3g	-	-	-	-	-	-	0.5g	-	-	15.7[5]
e.e.	n.m.	0.07	0.13	n.m.	0.27	0.36	0.99	1.00	n.m.	1.00	
c. 1M TRIS[2] pH = 7.5 additions	3g	-	-	-	-	0.5g	-	0.25g	-	-	15.2
e.e.	n.m.	0.18	0.40	n.m.	0.84	0.86[3]	0.94	0.95[3]	n.m.	0.83	
d. 1M TRIS[2] pH = 8.0 additions	3g	-	-	-	-	0.5g	-	0.5g	-		18.1
e.e.	n.m.	0.18	0.42	n.m.	0.78	0.88[3]	0.82	0.81[3]	n.m.	0.69	

n.m. = not measured
e.e. = enantiomeric excess

[1] MOPS = (3-N-morpholinopropane sulfonic acid)
[2] TRIS = Tris (hydroxymethyl) aminomethane
[3] Before addition of a new quantity of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol
[4] Incubation of cell suspensions a and c was continued with 50% of the original volume before addition of a new quantity of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol
[5] At 210 h
[6] A 40% extraction yield of the 1:1 ethyl acetate extraction for 2,2-dimethyl-1,3-dioxolane-4-methanol was assumed.

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Example 10

Rhodococcus erythropolis SCL 38—2 (CBS 179.86) was grown in a 10 l fermentor in 6 l of 5x concentrated GYMB medium. The medium was stirred, aerated (360 l/h), kept at a temperature of 30°C and a pH of 6.8 to 7.2. After 48 h of growth the culture, having an optical density of 30 was induced by a 5 h incubation with 10 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. The cells from the induced culture were harvested by centrifugation. A part of the obtained pellet was suspended in 1.5 l physiological salt solution (O.D. is 62) adjusted to a pH of 7.5 with 4N NaOH. The suspension was transferred to a 3 l fermentor vessel and incubated at 30°C, stirred and aerated with 90 l air/h. Addition of 1 g/l of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol resulted in acidification. Two feeds containing 4N NaOH and 8M *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol, respectively, were regulated automatically in the following way: the feeds ran with identical rate if the pH decreased below 7.5 and stopped if the pH increased above 7.5. The process continued during 99 h. Afterwards cells were spun down and resuspended in 1.5 l of fresh physiological salt solution and the feed of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol and NaOH were regulated identically as described for the first run, now during 142 h. A third run was done under similar conditions during 92 h except for the final 21 h, when only NaOH was supplied upon pH decrease below 7.5. The results presented in Table 6 show that resting cell suspensions can produce enantiomeric pure *R*-2,2-dimethyl-1,3-dioxolane-4-methanol.

TABLE 6

Concentrations and optical purities of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol and 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid

run	duration h	2,2-dimethyl-1,3-dioxolane-4-methanol g/l[1)]	ee (R-form)	2,2-dimethyl-1,3-dioxolane-4-carboxylic acid g/l[2)]
1	99	60.7	0.81	73.5
2	142	38.6	0.90	not measured
3	92	13.2	0.99	not measured

e.e. = enantiomeric excess

[1)] The concentration of 2,2-dimethyl-1,3-dioxolane-4-methanol was determined as in Example 7.

[2)] The concentration of 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid was determined after quantitative conversion of this acid to glyceric acid. This was done by an incubation of the fermentation broth containing 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid with $HClO_4$ during 1 h. After centrifugation the concentration of glyceric acid in the supernatant was assayed after elution of the supernatant over a HPLC cation exchanger (column: Aminex HPX 87 H Bio-Rad 125—0140).

Example 11

A 48 h old 6 l culture of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) obtained as described in Example 10 was incubated with 10 g/l of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 6 h.

The induced culture was supplied with 30 g of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol and after pH adjustment to 7.5 two feeds containing 4N NaOH and 8M *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol, respectively, were supplied upon every pH decrease below 7.5 as described in Example 10.

The process was continued during 52 h. The final broth contained 54.4 g/l of 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid in the form of the R-isomer (ee = 0.92). The concentration of the acid was assayed as described in Example 10.

The measurement of the enantiomeric purity of 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid was based on splitting of the $-CH_3$ signals in the NMR spectrum of the acid in the presence of an excess of R-(+)-1-(1-naphthyl) ethyl amine. Two methyl signals appear at 1.30 and 1.15 ppm respectively (due to the S-enantiomer) and two methyl signals appear at 1.10 and 1.05 ppm, respectively, (due to the R-enantiomer). The assignment of the $-CH_3$ resonances to *S*- and *R*-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid rests upon the ratio of converted *R*- and *S*-2,2-dimethyl-1,3-dioxolane-4-methanol.

The exact position of the $-CH_3$ resonances may vary with the amount of chiral solvating agent added.

Example 12

A 48 h old 6 l culture of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) obtained as described in Example 10 was incubated with 10 g/l of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 6 h.

After the incubation cells and the culture fluid were separated by centrifugation and the cells were resuspended in 6 l of physiological salt solution adjusted to pH = 7.5, which contained 15 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. Two feeds containing 4N NaOH and 8M *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol, respectively, were supplied during 29 h upon every pH decrease below 7.5, as described in Example 10.

The final cell suspension contained 27.9 g/l of 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid with an enantiomeric excess of 0.88. The concentration and optical purity of the acid were assayed as described in Example 11.

Example 13

Shake flasks of 2 l containing 500 ml GYMB medium were inoculated with 10 ml of a 24 h culture of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) grown on BHI medium in shake flasks. Cells were grown during 48 h at 30°C. The culture was induced by incubation in 5 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 6 h.

Cells originating from 1 liter of culture were resuspended in either 250 ml 1M MOPS buffer (pH = 7.5) or 250 ml 1M potassium phosphate buffer (pH = 7.5). The obtained suspensions were transferred to 2 l baffled shake flasks and *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was added at various time points. The concentrations of *R*- and *S*-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid were assayed as described in Example 11.

The results presented in Table 7 show that resting cell suspensions can produce large quantities of *R*-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid.

TABLE 7

Scheme of additions of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol and measured excess of the R enantiomer of the produced 2,2-dimethyl-1,3-dioxolane-4-carboxylic acid during incubation of cell suspensions in the presence of various buffers

Suspensions	time (h) 0	1	17	41	48	72	89	160	final conc. g/l
a. 1M MOPS pH = 7.5 additions	6.1 g	1.9 g	1.8 g	-	1.5 g	1.6 g	-	-	
e.e.	n.m.	n.m.	0.92	0.92	n.m.	n.m.	0.88	0.88	24.2
b. 1M potassium phosphate pH = 7.5 additions	6.1 g	2.0 g	1.9 g	-	-	-	-	-	
e.e.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	0.90	0.90	11.0

n.m. = not measured
e.e. = enantiomeric excess
MOPS = (3-N-morpholinopropane sulfonic acid)

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Example 14

Shake flask cultures of *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86) were grown on GYMB medium during 48 h and subsequently incubated with 5 g/l of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol during 5 h. The induced cultures were centrifuged and the cell pellet was divided in 4 parts. The parts A, B and C were suspended separately in physiological salt solution, one part D was suspended in physiological salt solution containing 10% glycerol. Suspension C was lyophilized. Samples A and C were incubated during 28 days at 4°C, B and D at −18°C. After the incubation sample C was resuspended in the original volume of physiological salt solution. The other suspensions were thawed. Cells of all obtained suspensions were spun down and resuspended in 100 ml of 1M MOPS (see Example 9) pH = 7.5 containing 10 g/l of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. The optical densities at 600 nm of all suspensions were 28. The suspensions were transferred to 500 ml baffled shake flasks and incubated in a shaker at 30°C. The increase of the enantiomeric excess of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol shows that the cells are still able to convert the S-enantiomer of this compound (see Table 8).

TABLE 8

Enantiomeric excess of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol in cell suspensions conserved in various ways

conservation temperature	further manipulations	ee of *R*-2,2-dimethyl-1,3-dioxolane-4-methanol after 1h	2h	3h	4h
T = 4°C	-	0.05	0.11	0.16	0.22
T = 4°C	lyophilized	0.06	0.10	0.14	0.21
T = -18°C	-	0.03	0.08	0.11	0.15
T = -18°C	10% glycerol	0.07	0.15	0.23	0.34

Example 15

The micro-organisms listed in Table 9 were grown for 48 hours at 30°C in 100 ml baffle flasks containing 25 ml of GYMB/MOPS medium. To a concentrated GYMB-medium a solution of MOPS [3-(N-morpholino)propane sulfonic acid], pH 7.0, was added, such that a 100 mM buffer was obtained in an original GYMB-medium.

To the grown cultures about 4.8 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol was added and the cultures were incubated further for 8, 24 and 48 hours. At each time point indicated two cultures of each micro-organism were extracted with an equal volume of ethyl acetate. By doing so an extraction recovery of about 40% of the 2,2-dimethyl-1,3-dioxolane-4-methanol present was obtained. The extracted compound from each culture separately was then derivatised and analysed on a chiral complexation column as described in Example 5.

The results are presented in Table 9.

Table 9

Incubation period	8 h			24 h			48 h		
Micro-organism	g/l*	%R	%S	g/l*	%R	%S	g/l*	%R	%S
Corynebacterium alkanum ATCC 21194	1.2	82	18	0.32	100	0	0.16	100	0
Corynebacterium equi A 2362 / CBS 264.87	2.2	53	47	0.95	99	1	0.86	99	1
Corynebacterium equi A 2431 / CBS 263.87	1.4	74	26	0.81	100	0	0.62	100	0
Corynebacterium hydrocarboclastus ATCC 15108	1.6	61	49	0.83	100	0	0.41	100	0
Corynebacterium sp. DS 5122 / CBS 265.87	1.9.	56	44	0.90	100	0	0.74	100	0
Nocardia canicruria ATCC 31548	0.91	100	0	0.50	100	0	0.27	100	0
Nocardia corallina ATCC 31338	1.7	59	41	0.85	100	0	0.45	100	0
Nocardia erythropolis T 487 / NCIB 9158	1.7	67	33	0.68	100	0	0.58	100	0
Nocardia erythropolis DSM 743	1.3	95	5	1.1	100	0	0.61	100	0
Nocardia erythropolis ATCC 4277	1.2	98	2	0.84	99	1	0.55	99	1
Nocardia paraffinae NCIB 11277	2.2	53	47	0.86	99	1	0.48	99	1
Nocardia spec. DS 5123 / CBS 266.87	1.8	63	37	0.98	100	0	0.72	100	0
Rhodococcus erythropolis SCL 38-2 / CBS 179.86	1.2	89	11	0.86	99	1	0.61	99	1
Rhodococcus rhodochrous NCIB 9703	1.2	88	12	0.59	99	1	0.24	99	1
Rhodococcus rhodochrous ATCC 21197	2.2	50	50	0.89	99	1	0.60	99	1

Table 9 (continued)

Incubation period	8 h			24 h			48 h		
Micro-organism	g/l*	%R	%S	g/l*	%R	%S	g/l*	%R	%S
Corynebacterium spec. T 1300 / CBS 267.87	n.d.	n.d.	n.d.	1.3	98	2	1.2	100	0
Mycobacterium rhodochrous / NCIB 11061	2.3	50	50	1.4	87	13	1.0	100	0
Nocardia aurantia NCIB 9557	0.91	100	0	0.84	100	0	0.47	100	0
Nocardia calcarea NCIB 8863	1.7	76	24	1.2	100	0	0.97	100	0
Nocardia cathaarde T 985 / CBS 268.87	2.0	53	47	n.d.	n.d.	n.d.	1.0	99	1
Nocardia globerula NCIB 9159	1.7	59	41	1.1	100	0	1.4	100	0
Nocardia ragosa NCIB 8926	2.4	54	46	0.63	99	1	0.54	100	0

n.d. = not determined.
All values presented are the mean of a duplicate result.
* The extracted quantities presented are *not* corrected for losses caused by a poor extraction recovery.

Example 16

The micro-organisms listed in Table 10 were grown as described in Example 15. However instead of *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol now about 2.4 or 4.8 g/l *R,S*-2,2-pentylene-4-hydroxymethyl-1,3-dioxolane (glycerol cyclohexanone derivative) was added to the grown cultures. The incubation proceeded for 24 or 48 hours. Extraction, derivatisation and analysis were as described in Examples 5 and 11.

The extraction recovery of the glycerol cyclohexanone derivative was estimated at 100%.

Preparation of 2,2-pentylene-4-hydroxymethyl-1,3-dioxolane

A stirred mixture of 100 ml (0.96 mole) of cyclohexanone, 130 ml (2.08 mole) of glycerol, 300 ml of hexane and 5 g of 4-toluenesulphonic acid monohydrate was refluxed while separating the condensed water layer for 20 hours. After the addition of 4 ml of triethyl amine, the mixture was concentrated in vacuum and the residue shaken with water/diethyl ether. The water layer was extracted with diethyl ether and the combined ether extracts were washed with 30% sodium chloride, dried with magnesium sulphate, filtered and evaporated to give 153 g of residue. This was vacuum distilled to give 113.6 g of title compound.

Table 10

Micro-organism	g/l substrate added	Incubation period 24 h			Incubation period 48 h		
		g/l extracted	%R	%S	g/l extracted	%R	%S
Corynebacterium equi A 2431 / CBS 263.87	4.8	n.d.			3.1	81	19
Nocardia erythropolis ATCC 4277	2.4	n.d			0.43	100	0
Rhodococcus equi IFO 03730	2.4	n.d			1.4*	94	6
Rhodococcus rhodochrous NCIB 9703	4.8	1.5	100	0	n.d.		
Rhodococcus rhodochrous ATCC 21197	2.4	n.d.			0.5	91	7

All values presented are the mean of a duplicate result except for * which value was obtained from a single experiment.
n.d. = not determined.

Example 17

The micro-organisms listed in Table 11 were grown as described in Examples 15 and 16. Now 2,2-butylene-4-hydroxymethyl-1,3-dioxolane-4-methanol (glycerol cyclopentanone derivative) was added to the grown cultures. The cultures were further incubated for 24 and 48 hours. Extraction, derivatisation and analysis were as described before.

The extraction recovery of the glycerol cyclopentanone derivative was estimated at 100%.

Preparation of 2,2-butylene-4-hydroxymethyl-1,3-dioxolane-4-methanol

A mixture of 88 ml (1 mole) of cyclopentanone, 140 ml (2.24 mole) of glycerol, 300 ml of hexane and 5 g of 4-toluenesulphonic acid monohydrate was stirred and refluxed while separating the condensed water layer for 28 hours. After cooling and the addition of 4 ml of triethyl amine the mixture was concentrated in vacuum and shaken with diethyl ether/water. The water layer was extracted with diethyl ether (3×) and the combined ether extracts were washed with water and brine, dried with magnesium sulphate and evaporated to give 117 g of residue. This was vacuum distilled to give 105 g of the title compound.

Table 11

Micro-organism	g/l substrate added	Incubation period 24 h			Incubation period 48 h		
		g/l extracted	%R	%S	g/l extracted	%R	%S
Corynebacterium alkanum ATCC 21194	4.8	1.6	88	12	0.66	91	9
Nocardia paraffinae NCIB 11277	4.8	n.d.			1.6	100	0
Rhodococcus erythropolis SCL 38-2 / CBS 179.86	2.4	0.57	100	0	n.d.		
Rhodococcus rhodochrous NCIB 9703	4.8	2.4	99	1	1.8	100	0
Rhodococcus rhodochrous ATCC 21197	4.8	n.d.			2.3	97	3

All values presented are the mean of a duplicate result.
n.d. = not determined.

Example 18

Rhodococcus rhodochrous strain NCIB 9703 and strain ATCC 21197 were grown as described in Examples 15 and 16. Now 2,2-diethyl-4-hydroxymethyl-1,3-dioxolane (glycerol pentanone derivative) was added to the grown culture and further incubated for 24 hours. Extraction, derivatisation and analysis were as described before.

The extraction recovery of the glycerol pentanone derivative was estimated at 100%.

The results are presented in Table 12.

Preparation of 2,2-diethyl-4-hydroxymethyl-1,3-dioxolane

A stirred mixture of 85 ml (0.805 mole) of 3-pentanone, 104 ml (1.68 mole) of glycerol, 250 ml of hexane and 4 g of 4-toluenesulphonic acid monohydrate was refluxed while separating the condensed water layer for 18 hours. After cooling 4 ml of triethyl amine was added and the mixture was concentrated in vacuum. The residue was shaken with diethyl ether/water. The water layer was extracted twice with diethyl ether and the combined extracts were washed with water and brine, dried with magnesium sulphate, filtered and evaporated to give 48 g of residue. This was vacuum distilled to give two fractions of 11.50 g and 6.12 g, respectively, of the title compound.

Table 12

Micro-organism	g/l substrate added	Incubation period 24 h g/l extracted	%R	%S
Rhodococcus rhodochrous NCIB 9703	2.4	0.8	100	0
	4.8	2.2	100	0
Rhodococcus rhodochrous ATCC 21197	2.4	1.5	70	30

All values presented are the mean of a duplicate result.

Example 19

Corynebacterium equi A 2431, CBS 263.87, *spec.* DS 5122, CBS 265.87 and *Nocardia spec.* DS 5123, CBS 266.87 were grown as described before in Example 15 and 16. Now 2.4 g/l of 4-hydroxymethyl-1,3-dioxolane (glycerol formaldehyde derivative) were then added to the grown cultures. The cultures were then further incubated for 6 hours. Thereafter the cells were separated from the broth by centrifugation and the supernatant eluted over an Exterlut-3-column (Merck nr. 15372). Thereafter the column was eluted with ethyl acetate for a GC-analysis. The extraction recovery using this procedure is estimated at 50%.

The results are presented in Table 13.

Table 13

Micro-organism	GC-analysis g/l extracted	%R	%S
Corynebacterium equi A 2431 / CBS 263.87	0.22	68	32
Corynebacterium spec. DS 5122 / CBS 265.87	0.15	82	18
Nocardia spec. DS 5123 CBS 266.87	0.035	100	0

Preparation of (*R,S*)-4-hydroxymethyl-1,3-dioxolane

A mechanically stirred mixture of 104 g (0.78 mole) of (*R,S*)-2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane, 500 ml of benzene, 86 g (1.53 mole) of powdered potassium hydroxide and 175 ml (1.52 mole) of benzyl chloride was refluxed for 17 hours while separating the condensed water layer (14 ml of water separated). After cooling the mixture was washed with water, 1 M sodium hydrogen carbonate and brine respectively, dried with magnesium sulphate, filtered and evaporated to give 287 g of crude benzyl ether. This was stirred with a mixture of 300 ml of methanol, 100 ml of water and 20 ml of 36% hydrochloric acid for 1,5 hours. Next the mixture was neutralized with about 10 g of sodium hydroxide in water and concentrated in vacuum. The residue was extracted with diethyl ether and the extract was dried with magnesium sulphate, filtered and evaporated to give 163 g of residue. This was purified over silica to give 118 g of (*R,S*)-3-benzyloxypropane-1,2-diol.

18.2 g (0.1 mole) of this was mixed with 50 ml of hexane, 10 g of paraformaldehyde and 0.5 g of paratoluenesulphonic acid monohydrate and stirred and refluxed for 4 hours while separating the condensed water layer. After cooling the mixture was washed with 1 M sodium hydrogen carbonate and brine, filtered and evaporated to give 17.78 g of *R,S*)-4-benzyloxymethyl-1,3-dioxolane. This was mixed with 100 ml of diethyl ether and 1.5 g of 10% palladium on carbon and stirred under atmospheric hydrogen for 4 hours. Next the catalyst was filtered off and the filtrate was evaporated and purified over silica with diethyl ether to give 8.07 g of the title compound.

Example 20

Rhodococcus erythropolis SCL 38—2 (CBS 179.86) and *Rhodococcus rhodochrous* ATCC 21197 were grown for 48 hours as described before (see Example 15). To some of the grown cultures about 4.8 g/l *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol were added and incubated further for about 5 hours, to induce the enzymatic activity normally responsible for the resolution of the *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. Cells from the induced culture were then harvested by centrifugation, washed with 100 mM MOPS-buffer pH 7.0 and collected twice concentrated in 100 mM of the same buffer and samples of 25 ml were put in 100 ml baffle flasks. About 5.0 g/l *R,S*-2,2-pentylene-4-hydroxymethyl-1,3-dioxolane (glycerol cyclohexanone derivative) were added to the suspensions and they were further incubated overnight at 30°C. The suspensions were then extracted and analysed as described before (see Example 15 and 16). The results presented in Table 14 show that the induced cells are able to convert stereospecifically the glycerol cyclohexanone derivative, whereas the non-induced cells do not or only very slowly attack the compound. A control experiment in which non-induced cells were also suspended and incubated with the two compounds mentioned showed that no degradation took place.

Table 14

Micro-organism	Conditions	Substrate: dimethyl derivative (1) g/l extracted	%R	%S	Substrate: cyclohexanone derivative (2) g/l extracted	%R	%S
	Normal assay (3) incubation period						
Rhodococcus erythropolis SCL 38-2 CBS 179.86	t = 5h	1.4	57	43			
	t = 24 h	0.96	100	0	5.4	51	49
	Induced cells in buffer. Incubation overnight.	0.90	100	0	3.2	81	19
	Normal assay (3) incubation period						
Rhodococcus rhodochrous ATCC 21197	t = 5h	2.2	52	48			
	t = 24 h	1.1	100	0	6.9	52	48
	Induced cells in buffer. Incubation overnight.	0.61	100	0	4.1	76	24

All values presented are the mean of a duplicate result.
(1) dimethyl derivative = *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. An extraction recovery of about 40% is assumed. The extracted quantities are not corrected for losses caused by a poor extraction recovery.
(2) cyclohexanone derivative = *R,S*-2,2-pentylene-4-hydroxymethyl-1,3-dioxolane. An extraction recovery of about 100% is assumed.
(3) Normal assay is the procedure as used in Example 15 and 16.

Example 21

A similar experiment as described in Example 20 was performed with *Nocardia canicruria* ATCC 31548, in which cells suspended with *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol were suspended in MOPS-buffer. Now, however, the substrate to be resolved, added to the suspensions, was *R,S*-2,2-diethyl-4-hydroxymethyl-1,3-dioxolane (glycerol pentanone derivative). The result of this experiment is presented in Table 15. (The same control experiment as described in Example 20 has also been performed using this substrate.)

Table 15

Micro-organism	Conditions	Substrate: dimethyl derivative (1) g/l extracted	%R	%S	Substrate: pentanone derivative (2) g/l extracted	%R	%S
	Normal assay (3) incubation period						
Nocardia canicruria ATCC 31548	t = 5h	1.3	72	28			
	t = 24 h	n.d. (4)	n.d.	n.d.	6.6	47	53
	Induced cells in buffer. Incubation overnight.	0.75	100	0	3.7	85	15

All values presented are the mean of a duplicate result.
(1) dimethyl derivative = *R,S*-2,2-dimethyl-1,3-dioxolane-4-methanol. An extraction recovery of about 40% is assumed. The extracted quantities are not corrected for losses caused by a poor extraction recovery.
(2) pentanone derivative = *R,S*-2,2-diethyl-4-hydroxymethyl-1,3-dioxolane.
(3) Normal assay is the procedure as used in Example 15 and 16.
(4) n.d. = not determined in this particular experiment.

Example 22

Corynebacterium equi A 2431 (CBS 263.87) and *Rhodococcus equi* (IFO 03730) were grown as described before in Example 15 and 16. Now 2.4 g/l of the asymmetric compound 2-methyl-2-isobutyl-4-hydroxymethyl-1,3-dioxolane (glycerol methyl isobutyl ketone derivative) were added to the grown cultures. The cultures were further incubated for 48 hours. Thereafter extraction was performed using methyl trichloride and the samples prepared for NMR-analysis. With NMR the four enantiomers could be resolved and the ratios between the enantiomers were determined. The signals originating from the two *R*-isomers (i.e. *R* for the configuration of the C_2 of the glycerol part of the derivative) could be denominated by using a glycerol methyl isobutyl ketone derivative synthesized from *R*-2,2-dimethyl-1,3-dioxolane-4-methanol as a reference compound.

The results are presented in Table 16. This example shows that asymmetric derivatives can also be resolved.

Table 16

Micro-organism	g/l extracted	percentage of the various enantiomers present R*/.. %	S*/.. %	R*/.. %	S*/.. %
Corynebacterium equi A 2431 /CBS 263.87	1.1	39	0	46	15
Rhodococcus equi IFO 03730	1.8	35	2	35	28

The values represented are the mean of a duplicate experiment.
* The first *R* or *S* denomination of the 4 enantiomers (R/R, R/S, S/R and S/S) concerns the C_2 of the glycerol part of the derivative. Which signal originates from the R/R enantiomer and which form the R/S was not determined.

Preparation of *R,S*-2-methyl-2-isobutyl-4-hydroxymethyl-1,3-dioxolane

A mixture of 65 ml (0.517 mole) of methyl isobutyl ketone, 200 ml of hexane, 70 ml (1.12 mole) of glycerol and 3 g of 4-toluenesulphonic acid monohydrate was stirred and refluxed while separating the condensed water layer for 48 hours. After cooling triethyl amine (4 ml) was added and the mixture was evaporated in vacuum and shaken with diethyl ether/water. The water layer was extracted (2x) with diethyl ether and the combined ether extracts were washed with water and brine, dried with magnesium sulphate, filtered and evaporated to give 70 g of residue. This was vacuum distilled to give 62 g of title compound.

Preparation of 2-methyl-2-isobutyl-4-(*R*)-hydroxymethyl-1,3-dioxolane

A stirred mixture of 5.48 ml (41.5 mmole) of (*R*)-(−)-2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane (e.e. = 0.9), 50 ml of benzene, 4.6 g of powdered potassium hydroxide and 10 ml of benzyl chloride was refluxed over molecular sieve 4A for 23 hours. After cooling the mixture was washed with water, 1 M sodium hydrogen carbonate and brine respectively, filtered and evaporated. The residue was stirred with a mixture of 15 ml of methanol, 5 ml of water and 1 ml of 36% hydrochloric acid for 1½ hours. Next the mixture was neutralized with sodium hydroxide, evaporated and extracted with diethyl ether. The extract was dried with magnesium sulphate, filtered and evaporated to give 9.40 g of oil. This was purified over silica to give 6.59 g of (*S*)-3-benzyloxy-propane-1,2-diol.

A mixture of 0.4 g of this, 10 ml of benzene, 1 ml of methyl isobutyl ketone and 30 mg of paratoluenesulphonic acid was refluxed for 18 hours over molecular sieve 4A. After cooling triethyl amine (50 ml) was added and the mixture was washed with water, filtered and evaporated. The residue was mixed with 10 ml of diethyl ether, 0.1 ml of triethyl amine and 0.2 g of 10% palladium on carbon and stirred under atmospheric hydrogen for 12 hours. The mixture was filtered and the filtrate was evaporated to give 0.34 g of the title compound, contaminated with the benzyl ether.

Example 23

Nocardia paraffinae NCIB 11277 was grown as described before in Example 15 and 16. Now 2.4 g/l of the asymmetric compound 2-methyl-4-hydroxymethyl-1,3-dioxolane (glycerol acetaldehyde derivative) were added to the culture and further incubated for 15 hours. The cells were then separated from the broth by centrifugation and the supernatant eluted over an Extrelut-3-column. Thereafter the column was eluted with ethyl acetate for a GC-analysis with which the four enantiomers could be separated. By using this procedure about 80% of the added substrate left over was extracted. As in Example 22 the *R*-isomers (i.e. *R* for the configuration of the C_2 of the glycerol part of the derivative) could be denominated by using a glycerol acetaldehyde derivative synthesized from *R*-2,2-dimethyl-1,3-dioxolane-4-methanol as a reference compound.

The result is presented in Table 17. As in Example 22 this result also shows that asymmetric derivatives can be resolved.

Preparation of *R,S*-2-methyl-4-hydroxymethyl-1,3-dioxolane (racemic glycerol acetaldehyde derivative)

A mixture of 70 ml of paraacetaldehyde, 400 ml of hexane, 200 ml of glycerol and 5.4 g of paratoluenesulphonic acid monohydrate was stirred and refluxed for 3 × 24 hours while separating the condensed water layer. After cooling triethyl amine (5 ml) was added and the mixture was concentrated in vacuum and the residue was vacuum distilled (65—84°C/1.5—3 mm Hg) giving 129.74 g of a mixture of the title compound and 2-methyl-5-hydroxy-1,3-dioxolane.

About 50 g of this was chromatographed twice over silica with diethyl ether as the eluent giving 9.45 g of racemic title compound, contaminated with 7.5% of 2-methyl-5-hydroxy-1,3-dioxolane.

Preparation of 2-methyl-4-(*R*)-hydroxymethyl-1,3-dioxolane (*R*-glycerol acetaldehyde derivative)

A mixture of 0.47 g (2.58 mmole) of (*S*)-3-benzyloxy-propane-1,2-diol, 10 ml of hexane, 0.4 ml of paraacetaldehyde and 15 mg of paratoluenesulphonic acid monohydrate was stirred and refluxed over molecular sieve 4A for 1.5 hours. After cooling a drop of triethyl amine was added and the mixture was washed with water, filtered and evaporated.

The residue was mixed with 10 ml of diethyl ether and 0.15 g of 10% palladium on carbon and stirred under atmospheric hydrogen for 3 hours. The mixture was filtered and evaporated to give 195 mg of the title compound.

Table 17

Micro-organism		percentage of the various enantiomers present			
	g/l extracted (1)	R*/.. % (2)	S*/.. % (2)	R*/.. % (2)	S*/.. % (2)
Nocardia paraffinae NCIB 11277	0.22	61	9	30	0

All values presented are the mean of a duplicate result.

(1) Not corrected for losses during recovery.

(2) The first *R* or *S* denomination of the 4 enantiomers (R/R, R/S, S/R and S/S) concerns the C_2 of the glycerol part of the derivative. Which GC-peak originates from the R/R enantiomer and which from the R/S was not determined.

Claims

1. Process for the preparation of a 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer wherein R_1 and R_2 are H or alkyl groups, optionally substituted or branched, or wherein R_1 and R_2 together with the carbon atom to which they are attached form a carbocyclic ring, optionally substituted, which comprises subjecting a mixture of *R*- and *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol to the action of a micro-organism, killed, alive or resting or extracts therefrom, optionally concentrated or purified, having the ability for stereoselective consumption of *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol for a period of time such that *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol in the mixture is consumed to give a 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in R-isomer.

2. Process according to claim 1 wherein the period of time is such that at least 80% of the *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is consumed.

3. Process according to claim 2 wherein the period of time is such that at least 90% of the *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is consumed.

4. Process according to any one of claims 1—3 wherein at least 99% of the R-isomer of 2,2-R_1,R_2-1,3-dioxolane-4-methanol is produced.

5. Process according to any one of claims 1—4 wherein the alkyl groups contain less than 6 carbon atoms or wherein the carbocyclic ring contains less than 8 carbon atoms.

6. Process according to any one of claims 1—5 wherein R_1 and R_2 are identical.

7. Process according to any one of claims 1—5 wherein R_1 and R_2 are H or an alkyl group containing 1—3 carbon atoms or together with the carbon atom to which they are attached form a carbocyclic ring containing 5 or 6 carbon atoms.

8. Process according to any one of claims 1—7 wherein said micro-organism is a bacterium, a yeast or a fungus.

9. Process according to any one of claims 1—7 wherein said micro-organism is a bacterium, preferably belonging to the genus *Nocardia,* to the genus *Rhodococcus,* to the genus *Corynebacterium,* or to the genus *Mycobacterium*..

10. Process according to claim 9 wherein the micro-organism used is *Rhodococcus equi,* preferably *Rhodococcus equi* (NCIB 12035) or *Rhodococcus equi* (IFO 03730).

11. Process according to claim 9 wherein the micro-organism used is *Rhodococcus rhodochrous,* preferably *Rhodococcus rhodochrous* (NCIB 9703) or *Rhodococcus rhodochrous* (ATCC 21197).

12. Process according to claim 9 wherein the micro-organism used is *Rhodococcus erythropolis,* preferably *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86), *Rhodococcus erythropolis* SCL 38—2R (CBS 180.86) or *Rhodococcus erythropolis* SCL 38—2S (CBS 181.86).

13. Process according to claim 9 wherein the micro-organism used is *Corynebacterium equi,* preferably *Corynebacterium equi* A 2362 (CBS 264.87) or *Corynebacterium equi* A 2431 (CBS 263.87).

14. Process according to claim 9 wherein the micro-organism used is *Corynebacterium alkanum* (ATCC 21194).

15. Process according to claim 9 wherein the micro-organism used is *Corynebacterium hydrocarboclastus* (ATCC 15108).

16. Process according to claim 9 wherein the micro-organism used is *Corynebacterium* sp. DS 5122 (CBS 265.87) or *Corynebacterium* sp. T 1300 (CBS 267.87).

17. Process according to claim 9 wherein the micro-organism used is *Nocardia erythropolis,* preferably *Nocardia erythropolis* T 487 (NCIB 9158), *Nocardia erythropolis* (DSM 743) or *Nocardia erythropolis* (ATCC 4277).

18. Process according to claim 9 wherein the micro-organism used is *Nocardia corallina* (ATCC 31338).

19. Process according to claim 9 wherein the micro-organism used is *Nocardia canicruria* (ATCC 31548).

20. Process according to claim 9 wherein the micro-organism used is *Nocardia paraffinae* (NCIB 11277).

21. Process according to claim 9 wherein the micro-organism used is *Nocardia spec.* DS 5123 (CBS 266.87).

22. Process according to claim 9 wherein the micro-organism used is *Nocardia aurantia* (NCIB 9557).

23. Process according to claim 9 wherein the micro-organism used is *Nocardia calcarea* (NCIB 8863).

24. Process according to claim 9 wherein the micro-organism used is *Nocardia cathaarde* T 985 (CBS 268.87).

25. Process according to claim 9 wherein the micro-organism used is *Nocardia globerula* (NCIB 9159).

26. Process according to claim 9 wherein the micro-organism used is *Nocardia ragosa* (NCIB 8926).

27. Process according to claim 9 wherein the micro-organism used is *Mycobacterium rhodochrous* (NCIB 11061).

28. Micro-organisms selected from the group consisting of
Rhodococcus erythropolis SCL 38—2 (CBS 179.86)
Rhodococcus erythropolis SCL 38—2R (CBS 180.86)
Rhodococcus erythropolis SCL 38—2S (CBS 181.86)
Corynebacterium equi A 2362 (CBS 264.87)
Corynebacterium equi A 2431 (CBS 263.87)
Corynebacterium sp. DS 5122 (CBS 265.87)
Nocardia spec. DS 5123 (CBS 266.87)
Corynebacterium sp. T 1300 (CBS 267.87) and
Nocardia cathaarde T 985 (CBS 268.87).

29. Process according to any one of the preceding claims wherein said micro-organism is immobilized either as a living cell, as a killed cell or as a resting cell.

30. Process according to any of the preceding claims, wherein the *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol is substantially converted into *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboxylic acid.

31. Use of 2,2-dimethyl-1,3-dioxolane-4-methanol enriched in R-isomer, prepared according to any one of the preceding claims, for the preparation of metoprolol enriched in S-isomer.

32. Process for the preparation of 2,2-R_1,R_2-1,3-dioxolane-4-methanol enriched in S-isomer comprising the conversion of *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboxylic acid, prepared according to the process according to claim 30, into *S*-2,2-R_1,R_2-1,3-dioxolane-4-methanol.

Patentansprüche

1. Verfahren zur Herstellung eines 2,2-R_1,R_2-1,3-Dioxolan-4-methanols, in dem das R-Isomere angereichert ist, wobei R_1 und R_2 für H oder gegebenenfalls substituierte oder verzweigte Alkylgruppen stehen oder wobei R_1 und R_2 zusammen mit dem Kohlenstoffatom, an das sie gebunden sind, einen gegebenenfalls substituierten carbocyclischen Ring bilden, dadurch gekennzeichnet, dass man ein Gemisch von R- und S-2,2-R_1,R_2-1,3-Dioxolan-4-methanol der Einwirkung eines Mikroorganismus, der

abgetötet, lebendig oder ruhend ist, oder von gegebenenfalls eingeengten oder gereinigten Extrakten davon mit der Fähigkeit zum stereoselektiven Verbrauch von S-2,2-R_1,R_2-1,3-Dioxolan-4-methanol aussetzt, und zwar während eines solchen Zeitraums, dass S-2,2-R_1,R_2-1,3-Dioxolan-4-methanol in dem Gemisch verbraucht wird unter Bildung eines 2,2-R_1,R_2-1,3-Dioxolan-4-methanols, in dem das R-Isomere angereichert ist.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass der Zeitraum derart ist, dass mindestens 80% des S-2,2-R_1,R_2-1,3-Dioxolan-4-methanols verbraucht werden.

3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, dass der Zeitraum derart ist, dass mindestens 90% des S-2,2-R_1,R_2-1,3-Dioxolan-4-methanols verbraucht werden.

4. Verfahren nach einem der Ansprüche 1 bis 3, dadurch gekennzeichnet, dass mindestens zu 99% das R-Isomere von 2,2-R_1,R_2-1,3-Dioxolan-4-methanol erzeugt wird.

5. Verfahren nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass die Alkylgruppen weniger als 6 Kohlenstoffatome enthalten oder dass der carbocyclische Ring weniger als 8 Kohlenstoffatome enthält.

6. Verfahren nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, dass R_1 und R_2 identisch sind.

7. Verfahren nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, dass R_1 und R_2 für H oder eine 1 bis 3 Kohlenstoffatome enthaltende Alkylgruppe stehen oder zusammen mit dem Kohlenstoffatom, an das sie gebunden sind, einen 5 oder 6 Kohlenstoffatome enthaltenden carbocyclischen Ring bilden.

8. Verfahren nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, dass der Mikroorganismus eine Bakterium, eine Hefe oder ein Pilz ist.

9. Verfahren nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, dass der Mikroorganismus ein Bakterium ist, das vorzugsweise zu der Gattung Nocardia, zu der Gattung Rhodococcus, zu der Gattung Corynebacterium oder zu der Gattung Mycobacterium gehört.

10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Rhodococcus equi, vorzugsweise Rhodococcus equi (NCIB 12035) oder Rhodococcus equi (IFO 03730), ist.

11. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Rhodococcus rhodochrous, vorzugsweise Rhodococcus rhodochrous (NCIB 9703) oder Rhodococcus rhodochrous (ATCC 21197), ist.

12. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Rhodococcus erythropolis, vorzugsweise Rhodococcus erythropolis SCL 38—2 (CBS 179.86), Rhodococcus erythropolis SCL 38—2R (CBS 180.86) oder Rhodococcus erythropolis SCL 38—2S (CBS 181.86), ist.

13. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Corynebacterium equi, vorzugsweise Corynebacterium equi A 2362 (CBS 264.87) oder Corynebacterium equi A 2431 (CBS 263.87), ist.

14. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Corynebacterium alkanum (ATCC 21194) ist.

15. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Corynebacterium hydrocarboclastus (ATCC 15108) ist.

16. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Corynebacterium sp. DS 5122 (CBS 265.87) oder Corynebacterium sp. T 1300 (CBS 267.87) ist.

17. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia erythropolis, vorzugsweise Nocardia erythropolis T 487 (NCIB 9158), Nocardia erythropolis (DSM 743) oder Nocardia erythropolis (ATCC 4277), ist.

18. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia corallina (ATCC 31338) ist.

19. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia canicruria (ATCC 31548) ist.

20. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia paraffinae (NCIB 11277) ist.

21. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia spec. DS 5123 (CBS 266.87) ist.

22. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia aurantia (NCIB 9557) ist.

23. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia calcarea (NCIB 8863) ist.

24. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia cathaarde T 985 (CBS 268.87) ist.

25. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia globerula (NCIB 9159) ist.

26. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Nocardia ragosa (NCIB 8926) ist.

27. Verfahren nach Anspruch 9, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Mycobacterium rhodochrous (NCIB 11061) ist.

28. Mikroorganismen, dadurch gekennzeichnet, dass sie aus der Gruppe gewählt sind, die aus
Rhodococcus erythropolis SCL 38—2 (CBS 179.86),
Rhodococcus erythropolis SCL 38—2R (CBS 180.86),
Rhodococcus erythropolis SCL 38—2S (CBS 181.86),
Corynebacterium equi A 2362 (CBS 264.87),
Corynebacterium equi A 2431 (CBS 263.87),
Corynebacterium sp. DS 5122 (CBS 265.87),
Nocardia spec. DS 5123 (CBS 266.87),
Corynebacterium sp. T 1300 (CBS 267.87) und
Nocardia cathaarde T 985 (CBS 268.87)
besteht.

29. Verfahren nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, dass der Mikroorganismus entweder als lebende Zelle, als abgetötete Zelle oder als ruhende Zelle immobilisiert wird.

30. Verfahren nach einem der vorangehenden Ansprüche, dadurch gekennzeichnet, dass das S-2,2-R_1,R_2-1,3-Dioxolan-4-methanol im wesentlichen in R-2,2-R_1,R_2-1,3-Dioxolan-4-carbonsäure übergeführt wird.

31. Verwendung von 2,2-Dimethyl-1,3-Dioxolan-4-methanol, in dem das R-Isomere angereichert ist, hergestellt nach einem der vorangehenden Ansprüche, für die Herstellung von Metoprolol, in dem das S-Isomere angereichert ist.

32. Verfahren zur Herstellung von 2,2-R_1,R_2-1,3-Dioxolan-4-methanol, in dem das S-Isomere angereichert ist, dadurch gekennzeichnet, dass man R-2,2-R_1,R_2-1,3-Dioxolan-4-carbonsäure, hergestellt mittels des Verfahrens nach Anspruch 30, in S-2,2-R_1,R_2-1,3-Dioxolan-4-methanol überführt.

Revendications

1. Procéde de préparation d'un 2,2-R_1,R_2-1,3-dioxolane-4-méthanol enrichi en l'isomère R, où R_1 et R_2 sont des H ou radicaux alcoyle éventuellement substitués ou ramifiés, ou bien R_1 et R_2, conjointement avec l'atome de carbone auquel ils sont attachés, forment un carbocycle éventuellement substitué, qui comprend l'exposition d'un mélange de *R*- et *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol à l'action d'un micro-organisme tué, vivant ou au repos ou d'extraits de celui-ci, éventuellement concentrés ou purifiés, ayant l'aptitude à la consommation stéréosélective du *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol pendant une durée telle que le *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol du mélange soit consommé pour donner un 2,2-R_1,R_2-1,3-dioxolane-4-méthanol enrichi en l'isomère R.

2. Procéde suivant la revendication 1, dans lequel la durée est telle que le *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol soit consommé pour au moins 80%.

3. Procéde suivant la revendication 2, dans lequel la durée est telle que le *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol soit consommé pour au moins 90%.

4. Procéde suivant l'une quelconque des revendications 1 à 3, dans lequel l'isomère R du 2,2-R_1,R_2-1,3-dioxolane-4-méthanol est produit pour au moins 99%.

5. Procédé suivant l'une quelconque des revendications 1 à 4, dans lequel les radicaux alcoyle comptent moins de 6 atomes de carbone ou dans lequel le carbocycle compte moins de 8 atomes de carbone.

6. Procédé suivant l'une quelconque des revendications 1 à 5, dans lequel R_1 et R_2 sont identiques.

7. Procédé suivant l'une quelconque des revendications 1 à 5, dans lequel R_1 et R_2 sont H ou un radical alcoyle comptant 1 à 3 atomes de carbone ou, conjointement avec l'atome de carbone auquel ils sont attachés, forment un carbocycle comptant 5 ou 6 atomes de carbone.

8. Procédé suivant l'une quelconque des revendications 1 à 7, dans lequel le micro-organisme est une bactérie, une levure ou un champignon.

9. Procédé suivant l'une quelconque des revendications 1 à 7, dans lequel le micro-organisme est une bactérie, appartenant de préférence au genre *Nocardia*, au genre *Rhodococcus*, au genre *Corynebacterium*, ou au genre *Mycobacterium*..

10. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Rhodococcus equi*, de préférence *Rhodococcus equi* (NCIB 12035) ou *Rhodococcus equi* (IFO 03730).

11. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Rhodococcus rhodochrous*, de préférence *Rhodococcus rhodochrous* (NCIB 9703) ou *Rhodococcus rhodochrous* (ATCC 21197).

12. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Rhodococcus erythropolis*, de préférence *Rhodococcus erythropolis* SCL 38—2 (CBS 179.86), *Rhodococcus erythropolis* SCL 38—2R (CBS 180.86) ou *Rhodococcus erythropolis* SCL 38—2S (CBS 181.86).

13. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Corynebacterium equi*, de préférence *Corynebacterium equi* A 2362 (CBS 264.87) ou *Corynebacterium equi* A 2431 (CBS 263.87).

14. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Corynebacterium alkanum* (ATCC 21194).

15. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Corynebacterium hydrocarboclastus* (ATCC 15108).

16. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Corynebacterium* sp. DS 5122 (CBS 265.87) ou *Corynebacterium* sp. T 1300 (CBS 267.87).

17. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia erythropolis*, de préférence *Nocardia erythropolis* T 487 (NCIB 9158), *Nocardia erythropolis* (DSM 743) ou *Nocardia erythropolis* (ATCC 4277).

18. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia corallina* (ATCC 31338).

19. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia canicruria* (ATCC 31548).

20. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia paraffinae* (NCIB 11277).

21. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia spec.* DS 5123 (CBS 266.87).

22. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia aurantia* (NCIB 9557).

23. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia calcarea* (NCIB 8863).

24. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia cathaarde* T 985 (CBS 268.87).

25. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia globerula* (NCIB 9159).

26. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Nocardia ragosa* (NCIB 8926).

27. Procédé suivant la revendication 9, dans lequel le micro-organisme utilisé est *Mycobacterium rhodochrous* (NCIB 11061).

28. Micro-organismes choisis dans la classe formée par
Rhodococcus erythropolis SCL 38—2 (CBS 179.86)
Rhodococcus erythropolis SCL 38—2R (CBS 180.86)
Rhodococcus erythropolis SCL 38—2S (CBS 181.86)
Corynebacterium equi A 2362 (CBS 264.87)
Corynebacterium equi A 2431 (CBS 263.87)
Corynebacterium sp. DS 5122 (CBS 265.87)
Nocardia spec. DS 5123 (CBS 266.87)
Corynebacterium sp. T 1300 (CBS 267.87) et
Nocardia cathaarde T 985 (CBS 268.87).

29. Procéde suivant l'une quelconque des revendications précédentes, dans lequel le micro-organisme est immobilisé à l'état de cellule vivante, de cellule tuée ou de cellule au repos.

30. Procédé suivant l'une quelconque des revendications précédentes, dans lequel *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol est en substance converti en acide *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboxylique.

31. Utilisation du 2,2-diméthyl-1,3-dioxolane-4-méthanol enrichi en l'isomère R, préparé suivant l'une quelconque des revendications précédentes, pour la préparation du métoprolol enrichi en l'isomère S.

32. Procédé de préparation du 2,2-R_1,R_2-1,3-dioxolane-4-méthanol enrichi en l'isomère S, comprenant la conversion de l'acide *R*-2,2-R_1,R_2-1,3-dioxolane-4-carboxylique, préparé par le procédé suivant la revendication 30, en *S*-2,2-R_1,R_2-1,3-dioxolane-4-méthanol.

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/135

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [6]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0339006 (ASTRA) 25 October 1989, see page 2, lines 1-24; claims	1-6
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Y		7

Y	Patent Abstracts of Japan, vol. 15, no. 128 (C-818)[4656], 28 March 1991, & JP,A,311014 (NISSHIN OIL MILLS LTD) 18 January 1991, see the entire document	7
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[6] Special categories of cited documents : [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15-07-1992	26.08.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	GOETZ G.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9201950 SA 59840

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 14/08/92
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		SE-A-	8504721	12-04-87
		US-A-	4957745	18-09-90
US-A- 4818541	04-04-89	None		

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Published
With international search report.

(54) Title: NEW MANUFACTURING PROCESS OF METOPROLOL

(57) Abstract

A method for the manufacture of metoprolol wherein the process is performed in water as solvent.

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NEW MANUFACTURING PROCESS OF METOPROLOL

Field of the invention

The present invention relates to an improved method for the manufacture of metoprolol base 1-(isopropylamino)-3-[p-(2-methoxyethyl)-phenoxy]-2-propanol) via the route of reacting p-(2-methoxyethyl)-phenol (A) and epichlorohydrin (B) and then reacting the obtained 1-(2,3-epoxypropoxy)-4-(2-methoxyethyl)-benzene (AB) with isopropylamine (C). The crude metoprolol base is then purified.

Prior art

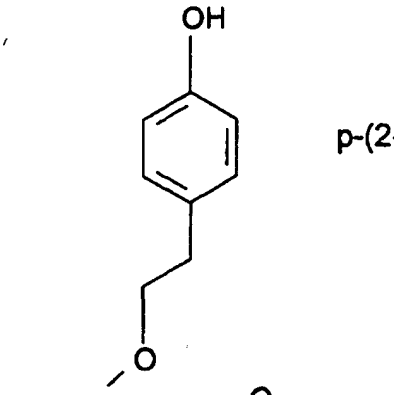
Chemical Abstracts, vol. 112 (1990) abstract no 197820 discloses the reaction of p-(2-methoxyethyl)-phenol and epichlorohydrin in the two phase system of water and organic solvent.

Swedish patents 354 851 and 368 004 disclose the reaction of p-(2-methoxyethyl)-phenol and epichlorohydrin where the epichlorohydrin is used not only as a building block in the reaction but also as solvent.

Disclosure of the invention

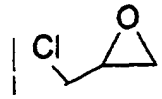
It has now been found that metoprolol can be prepared in a manner that is fast, environmentally sound and gives a good yield and high purity using reactants that are known per se. The difference from the prior art is that the new method uses no other solvents than water for the reaction of A and B. From an environmental as well as an occupational hazard point of view it is a great advantage to be able to replace a hazardous organic solvent with an non-noxious solvent such as water.

The method of the invention is illustrated by the reaction scheme below:

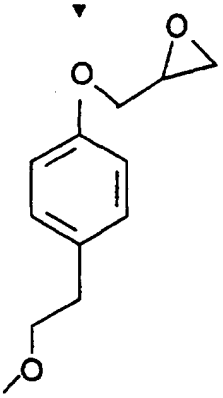


p-(2-Methoxyethyl)phenol (A)

Step 1

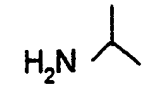


Epichlorohydrin (B)

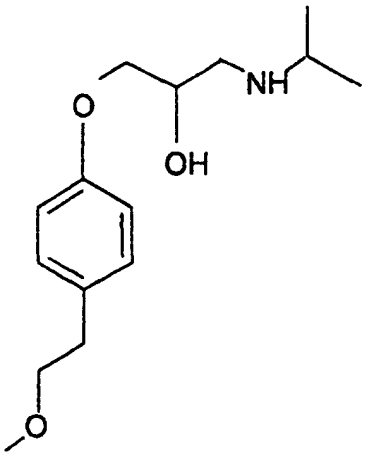


1-(2,3-Epoxypropoxy)-4-
(2-methoxyethyl)-benzene (AB)

Step 2



Isopropylamine (C)



1-Isopropylamino-3-[p-(2-
methoxyethyl)-phenoxy]
2-propanol, metoprolol base

General example

p-(2-Methoxyethyl)phenol (A) and epichlorohydrin (B, 1.4-2.0 eqv.) are reacted in water, at least 1.5 kg, preferably about 2 kg of water per kg of phenol, during the addition of sodium (or potassium) hydroxide solution, (1.3-1.7 eqv.) to form 1-(2,3-epoxypropoxy)-4-(2-methoxyethyl)benzene; (p-methoxyethyl-epoxypropoxybenzene). The reaction is preferably performed at a temperature of 50 - 70 °C.

The two phases are separated, and the p-methoxyethylepoxy-propoxybenzene is isolated by distillation under reduced pressure. More particular, the excess of epichlorohydrin is evaporated, and the epoxide is distilled under reduced pressure to obtain a product with a purity of about 96-98%. If desired, before distilling the main fraction of the epoxide, a prefraction/forecut (2-8 %, preferably 4-6 %) thereof could be distilled. The isolation by destillation of the epoxide under reduced pressure is an important part of the process and essential for the quality of the end product.

The epoxide is reacted with isopropylamine preferably in isopropyl alcohol to form metoprolol base. The amount of isopropylamine in relation to epoxide is at least 1 eqvivalent, preferably 3-6 eqvivalents. The reaction mixture is then treated in order to eliminate the excess of isopropylamine.

Alternatively, the amination with isopropylamine is carried out in a pressurized system without isopropyl alcohol at 70 ± 10°C at pressures of 2.8-3.2 kg / 275-315 kPa.

The resulting metoprolol is dissolved in toluene, isobutyl methyl ketone or butyl acetate and extracted with dilute hydrochloric acid or sulphuric acid, preferably at pH 4-6. The phases are separated and the chosen solvent with sodium or potassium hydroxide solution to adjust the pH to 11 - 13 is added to the aqueous phase. The two phases are separated, and the organic phase is evaporated in vacuo to an oily residue of metoprolol base which is dissolved in acetone. Purified metoprolol base is then obtained by conventional means.

Working example

1-(2,3-epoxypropoxy)-4-(2-methoxyethyl) benzene

p-(2-Methoxyethyl)phenol (A, ~ 6,6 mol), epichlorohydrin (B, 1.45 eqv.) and water (~ 2 kg) were combined and the mixture heated to ~ 50°C.
Sodium hydroxide solution (50%; 1.4 eqv.) was added during 3 hours and the temperature was elevated to reach approximately 60°C during the addition. Formation of the title compound occured during this period.

The batch was stirred for another hour at approximately 60°C, then cooled to approximately 50°C and the phases were separated and the product washed with water.

The residue was distilled at ≤190°C and a pressure of ≤20 mm Hg and the distillate was collected. The yield of the title compound was 80% of theory and the purity was 98% according to GC analysis.

Metoprolol base

1-(2,3-epoxypropoxy)-4-(2-methoxyethyl)benzene (1 kg, 4.8 mol), isopropyl alcohol (~0.9 kg) and isopropylamine (0.8-1.7 kg, 3-6 eqv.) were mixed and reacted for 2-5 hours at reflux. Formation of metoprolol base occured during this period.

The reaction mixture was then concentrated at atmospheric pressure until the inner temperature reached ~100°C. Water was added to the batch and then distilled off in vacuo until the inner temperature reached ~100°C to form a concentrate.

The resulting concentrate was diluted with isobutyl methyl ketone (~0.6 kg) and water (~2.2 kg), and concentrated sulphuric acid was added, to adjust the pH to 4-6.

After separation, isobutyl methyl ketone (~ 1 kg) was added to the water layer, and concentrated sodium hydroxide solution was added to adjust the pH to 13.

The organic layer was concentrated in vacuo at ≤80°C, until distillation ceased, and the concentrated batch was redissolved in acetone (~1.6 kg) and filtered, to yield metoprolol base solution. The assay of metoprolol base in the solution was determined by titration. Yield: ~1.2 kg metoprolol base (100 %) ~95 % of theory. The purity of the metoprolol base was 96 %.

CLAIMS

1. A method for the manufacture of metoprolol, characterized by reacting in a first step p-(2-methoxyethyl)phenol and epichlorohydrin in water as solvent and at a temperature of 50 to 70°C, evaporating the excess of epichlorohydrin and then distilling the obtained 1-(2,3-epoxypropoxy)-4-(2-methoxyethyl)-benzene under reduced pressure, and in a second step reacting the obtained 1-(2,3-epoxypropoxy)-4-(2-methoxyethyl)-benzene and isopropylamine is the presence of isopropyl alcohol to form metoprolol base.

2. A method according to claim 1 wherein in the first step is carried out in the presence of sodium hydroxide.

3. A method according to claim 1 wherein in the first step is carried out in the presence of potassium hydroxide.

4. A method according to any of the preceding claims wherein the resulting metoprolol base is purified by dissolving the metoprolol base in a solvent selected among toluene, isobutyl methyl ketone and butyl acetate and extracted with either hydrochloric or sulphuric acid solution.

5. Metoprolol as prepared by the process according to any of claims 1-4.

6. A method as claimed in any one of claims 1 to 4 wherein the resulting metoprolol is converted into metoprolol tartrate.

7. A method as claimed in any one of claims 1 to 4 wherein the resulting metoprolol is converted into metoprolol succinate.

8. A method for the manufacture of a pharmaceutical preparation wherein metoprolol is produced by the method as claimed in any one of claims 1 to 4 or 6 to 7 and the metoprolol is thereafter formulated with a pharmaceutically acceptable diluent or carrier.

9. A method as claimed in claim 8 wherein a diuretic is included in the ingredients for the pharmaceutical preparation.

10. A method as claimed in claim 9 wherein the diuretic is hydrochlorothiazide.

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(54) Title: COMPOSITION AND METHOD CONTAINING OPTICALLY PURE (S) METOPROLOL

(57) Abstract

Optically pure (S) metoprolol, which is substantially free of the (R) enantiomer, is a potent beta-blocker for treating myocardial infarction and for relieving the symptoms of angina pectoris, cardiac arrhythmia and hypertension in individuals. A method is disclosed utilizing the optically pure (S) configurational enantiomer of metoprolol for treating cardiovascular disorders while reducing undesirable side effects associated with the administration of beta-blockers.

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COMPOSITION AND METHOD CONTAINING OPTICALLY PURE (S) METOPROLOL

Description

Background

Metoprolol is a drug belonging to the general class of compounds known as beta-blockers. Beta-blockers are beta-selective adrenoreceptor blocking agents, and include well-known commercial products such as propanolol and atenolol. Several members of this drug class are known to be useful in treatment of hypertension, angina pectoris, and myocardial infarction.

Although the mechanism of the antihypertensive effect of metoprolol (and other β-blockers) is not known with certainty, a number of mechanistic possibilities have been advanced, including: the suppression of endogenous catecholamines at cardiac adrenergic neuron sites; a central effect leading to reduced sympathetic outflow to the periphery; and suppression of rennin activity.

The effectiveness of metoprolol in treatment of angina pectoris is likely to be associated with its tendency to reduce the oxygen requirements of the heart at various levels of effort. This effect results from blockage of catecholamine-induced increases in heart rate, blood pressure, and in velocity and extent of myocardial contraction.

Metropolol is regarded to be a relatively selective β-blocker. That is, it has a preferential effect on β1 adrenoreceptors which are predominant in cardiac muscle. This selectivity is not absolute, however, and metoprolol also exhibits activity on β2 adrenoreceptors located in bronchial and peripheral vascular tissue.

Metropolol is a racemic mixture. That is, it is a mixture of optical isomers, called enantiomers. Enantiomers are structurally similar compounds which differ only in that one isomer is a configurational mirror image of the other and the mirror images cannot be superimposed. This phenomenon is known as chirality. Although structurally similar, enantiomers can have profoundly different effects in biological systems; one enantiomer is often biologically active while the other has little or no biological activity at all.

Summary of the Invention

The present invention relates to a method of treating cardiovascular disorders, including angina pectoris, cardiac arrhythmia, hypertension or myocardial infarction in an individual comprising administering to the individual a therapeutic amount of the (S) enantiomer of metoprolol which is substantially free of the (R) enantiomer. The method is useful in treating cardiovascular disorders while reducing or avoiding undesirable side effects such as: central nervous system effects (tiredness, dizziness, short-term memory loss, headache,

nightmares and insomnia); cardiovascular effects (bradycardia, cardiac depression, cold extremities, palpitations, and peripheral edema); respiratory effects (shortness of breath, wheezing, dyspnea); hypersensitive reactions (pruritis, rash); and miscellaneous effects such as vertigo, decreased libido and hallucinations which are associated in whole or in part with the (R) enantiomer. For beta-blocking drugs, it is important to have a beta-blocking composition which also minimizes these side effects. A composition containing the (S) isomer of metoprolol is particularly useful because the (S) isomer exhibits both of these desired characteristics.

Of particular importance is the fact that for patients suffering from cardiac failure along with hypertension, or angina pectoris, further cardiac depression caused by bradycardia and decreased myocardial contractility can lead to a worsening of their overall condition. Also these latter effects can lead gradually to cardiac failure in patients who have not exhibited this problem. In the method of the present invention, bradycardia and decreased myocardial contractility are less pronounced than when metoprolol is administered as the racemic mixture.

The present method provides a safe, highly effective method for treating the cardiac disorders associated with hypertension, angina pectoris or myocardial infarction.

Detailed Description of the Invention

The present invention relies on the beta-blocking activity of the S configurational enantiomer of metoprolol , referred to as (S) metoprolol , to provide enhanced beta-blocking activity, for example, in treatment of hypertension, angina pectoris or myocardial infarction, without many of the undesirable side effects associated with beta-blockers, i.e., central nervous system effects, cardiovascular effects, respiratory effects, and miscellaneous effects such as vertigo, decreased libido and hallucinations. In the present method, (S) metoprolol, which is substantially free of its (R) enantiomer, is administered alone, or in combination with other drugs in adjunctive treatment, to an individual suffering from a cardiovascular disorder, such as heart disease, angina or hypertension. "(S) metoprolol" as used herein refers to the S configurational isomer of 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol, and also pharmaceutically acceptable salts, such as the tartrate salt, of the compound. The term "substantially free of the (R) enantiomer" as used herein means that the composition contains at least 90% by weight (S) metoprolol and 10% by weight or less of (R) metoprolol.

Prior to this invention, metoprolol has been administered as the racemic mixture. However, by the method of the present invention (S) metoprolol is administered substantially free of the (R) enantiomer. (R) metoprolol can contribute to adverse

side effects in some individuals without any desired therapeutic effect. Thus, it is desirable to use the pure (S) isomer in cardiovascular applications, because it is much more cardioactive than the (R) isomer, and because it minimizes activity associated with the undesirable side effects of the (R) isomer.

In the present method, (S) metoprolol is administered to an individual suffering from a cardiovascular disorder, such as angina pectoris, cardiac arrhythmia, hypertension or myocardial infarction. For example, (S) metoprolol is administered therapeutically to an individual after a heart attack, or to reduce or ameliorate hypertension and regulate heart beat or to reduce the symptoms of angina pectoris. Alternatively, (S) metoprolol can be administered prophylactically to reduce the probability of occurrence of a heart attack.

Metroprolol, like propranolol and certain other beta-blockers, undergoes substantial first-pass metabolism by the liver before entering the circulatory system. Approximately 40-50% of the administered oral dose appears intact in the systemic circulation. Shetty and Nelson (J. Med. Chem., 31, 55-59, 1988) have studied the stereochemical aspects of the metabolic processing of metoprolol and demonstrated that the primary metabolic process, benzylic hydroxylation, is effected stereoselectively. Both enantiomers of metoprolol are hydroxylated to form metabolites of the (R) configuration at the new benzylic (1') chiral center. (S) metoprolol is hydroxylated with a significantly higher degree of

stereoselectivity than (R) metoprolol (R/S ratio at the new benzylic (1') chiral center of 26 vs. 9.4).

The high degree of first-pass metabolism of metoprolol requires the administration of higher doses by the oral route than would otherwise be required. One aspect of the present invention, therefore, is that the (S) metoprolol enantiomer is sufficiently potent that it can be administered by means that avoid such first-pass metabolism (for example transdermally), thereby resulting in a systemic concentration in the therapeutically effective range with a substantial reduction in the needed dosage. Racemic metoprolol is not conveniently administered in such forms, because of the substantially higher doses involved.

Delivery methods that offer such advantages include but are not limited to transdermal patches, topical creams and ointments, electrically-stimulated transdermal delivery systems and metered injection delivery systems.

Other possible routes of drug administration are orally, by subcutaneous or other injection, intravenously, parenterally, rectally or via by sustained release methods, e.g., an implanted reservoir containing (S) metoprolol. The form in which the drug will be administered (e.g., powder, tablet, capsule, solution, emulsion) will depend on the route by which it is administered. The quantity of the drug to be administered will be determined on an individual basis, and will be based at least in part on consideration of the individual's size, the

severity of the symptoms to be treated and the result sought. In general, quantities of (S) metoprolol sufficient to treat hypertension, angina pectoris, or myocardial infarction will be administered.

Racemic metoprolol is usually administered in doses of 100-450 mg per day, in single or divided doses. In the method of the present invention, doses of (S) metoprolol are in the range of 50-300 mg daily when administered orally. Dosages of 2-100 mg daily may be admininstered by methods which avoid first-pass hepatic metabolism, and preferably doses in the range of 5-25 mg daily.

In the method of the present invention, (S) metoprolol can be administered along with one or more other drugs. For example, other anti-hypertensive agents, such as thiazide-type diuretics, hydralazine, prazosin, and alpha-methyl dopa, can be given with or in close temporal proximity to administration of (S) metoprolol. The two (or more) drugs ((S) metoprolol and another drug) can be administered in one composition or as two separate entities. For example, they can be administered in a single capsule, tablet, powder, liquid, etc. or as individual compounds. The components included in a particular composition, in addition to (S) metoprolol and another drug or drugs, are determined primarily by the manner in which the composition is to be administered. For example, a composition to be administered orally in tablet form can include, in addition to the drugs, a filler (e.g., lactose), a binder (e.g., carboxymethyl cellulose, gum arabic,

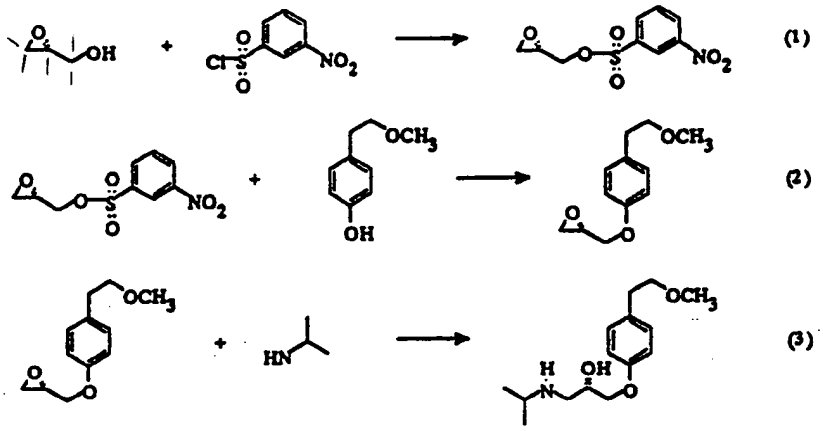
gelatin), an adjuvant, a flavoring agent, a coloring agent and a coating material (e.g., wax or a plasticizer). A composition to be administered in liquid form can include the combination of drugs and, optionally, an emulsifying agent, a flavoring agent and/or a coloring agent.

In general, according to the method of the present invention, (S) metoprolol, alone or in combination with another drug(s), is administered to an individual periodically as necessary to reduce or ameliorate symptoms of hypertension, angina pectoris or myocardial infarction while reducing or avoiding undesirable side effects associated with beta-blockers, including cardiac, central nervous system and respiratory effects. The length of time during which the drugs are administered and the dosage will depend on the disorder being treated, the type and severity of the symptoms, and the physical condition of the individual being treated.

The invention is further illustrated by the following example. This example is not intended to be limiting of the invention in any way.

EXAMPLE 1

PREPARATION OF (S) METOPROLOL



Preparation of S-Glycidyl m-Nitrobenzenesulfonate (reaction 1):

A solution of R-glycidol and triethylamine in toluene was cooled with ice water (ca. 5°C). m-Nitrobenzenesulfonyl chloride was added in portions while maintaining the temperature below 10°C. During the addition, a white precipitate (triethylamine hydrochloride) was formed. The mixture was stirred at room temperature for 22 hours. The mixture was then diluted with a small volume of ethyl acetate and filtered. The solid residue was washed thoroughly with ethyl acetate. The filtrate was then concentrated to dryness to give a yellow oil which on

standing and cooling became a solid. The solid was recrystallized twice from ethyl acetate/hexane until the optical rotation did not change.

Preparation of p-(S-Glycidyloxy)-Methoxyethylbenzene (reaction 2):

Dry dimethylformamide (DMF) is cooled to ca. 5°C with ice-water. Potassium t-butoxide is then added to form a solution. p-Hydroxy-methoxyethyl-benzene in dry DMF is then slowly added at 5-10°C with vigorous stirring. The resulting mixture is then warmed to room temperature and stirred for a number of hours before being cooled back to 5°C with ice-water. S-Glycidyl m-nitrobenzenesulfonate in DMF is then slowly added. After the addition, the mixture is stirred while allowing it to warm to room temperature. The reaction is then adjusted to neutral pH with NaH_2PO_4. The mixture is filtered and the residue washed with DMF. The combined filtrate is then concentrated in vacuo and poured into saturated NaCl solution at 5°C with stirring. The resulting solid is collected by filtration and dried to give the title compound as a wet solid. The product is used without further purification in the next reaction.

Preparation of (S) Metoprolol (reaction 3):

The wet solid from the above reaction is added to water followed by isopropylamine. The suspension is heated to reflux and stirred for a number of hours. The reaction is followed by HPLC. The

reaction is then cooled to room temperature and the excess isopropylamine removed by distillation. The resulting mixture is then saturated with NaCl. A solution of NaOH is then added to make the mixture 2% in NaOH. The mixture is stirred, then cooled to 5°C and filtered. The solid is washed with water and dried in vacuo to give the title compound.

CLAIMS

1. Use of (S) metoprolol, which is substantially free of (R) metoprolol, for the manufacture of a medicament for the treatment of a cardiovascular disorder and, at the same time, for reducing undesirable side effects associated with racemic metoprolol, wherein the treatment alleviates the cardiovascular disorder.

2. The use of Claim 1 wherein the cardiovascular disorder is hypertension, cardiac arrhythmia, angina pectoris or myocardial infarction.

3. The use of Claim 1 wherein the amount of (S) metoprolol is greater than about 90% by weight of the metoprolol portion.

4. The use of Claim 3 wherein the amount of (S) metoprolol is greater than about 99% by weight of the metoprolol portion.

5. The use of Claim 1 for the manufacture of a medicament wherein the amount of (S) metoprolol is sufficient to reduce, ameliorate or eliminate the symptoms of the cardiovascular disorder and, at the same time, reduce or eliminate the undesirable side effects associated with racemic metoprolol.

6. The use of Claim 1 for the manufacture of a medicament in unit dosage form having up to about 300 mg of (S) metoprolol.

7. The use of Claim 1 for the manufacture of a medicament wherein the (S) metoprolol is in a delivery means that is a transdermal patch, topical cream, topical ointment, an electrically-stimulated transdermal delivery system or a metered injection delivery system.

8. Use of (S) metoprolol and at least one additional drug for the manufacture of a medicament for the treatment of a cardiovascular disorder and, at the same time, for reducing undesirable side effects associated with racemic metoprolol, wherein the treatment alleviates the cardiovascular disorder.

9. The use of Claim 8 wherein the additional drug is an anti-hypertensive agent.

10. The use of Claim 9 wherein the additional drug is a thiazide-type diuretic, hydralazine, prazosin or alpha-methyldopa.